Habitat ecology and microclimatic influences on the distribution of *Ixodes ricinus* ticks in Baden-Württemberg

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von

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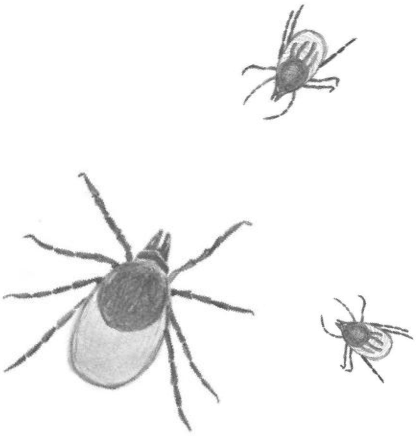
Dedicated to a family

whose lives were changed dramatically

through the bite of a single tick.



Your fate was the initial spark
that enlightend my personal desire to work in this research field.
It strongly motivated my search for a deeper understanding of this species in hoping to contribute to the protection of people in the future.

ABSTRACT

The tick species *Ixodes ricinus* is currently the most relevant transmitter of diseases in central Europe. In Baden-Württemberg, it is particularly well known for transmitting tick-borne encephalitis and borreliosis to humans. The risk of getting a tick-borne disease increases along with the probability of a tick bite and thereby with the number of ticks. However, to date it is not possible to estimate the abundance of ticks in a specific habitat by the habitats suitability, based on relevant environmental factors. Therefore, the aim of this study was to create a set of useful methods, key factors and concepts which allow the identification of a habitat's suitability for *Ixodes ricinus* ticks in its regional setting, on the example of Baden-Württemberg.

From fall 2012 to fall 2015, a total of 22,697 *I.ricinus* ticks were sampled, a variety of microclimatic raw data were recorded continuously on-site and data concerning vegetation and soil properties were collected at 25 forest habitats across Baden-Württemberg. To evaluate this comprehensive dataset, a high variety of analysis approaches was used: partially standard methods in tick research; some methods were tailored from other disciplines and others were devised specifically on the basis of knowledge about the ticks' physiology to suit the purposes. In addition, the habitat type "forest" was deconstructed to fundamental, ecological processes and patterns relevant to the ticks' needs.

The data analysis presented in this work provided several novel findings. Humus is a key factor for a tick's water provision and thus tick density in a given habitat. The humus factor influences also the duration of a tick's development and very likely even the activity start in spring. The effect of a single environmental factor on tick abundances have found to vary from year to year and from habitat to habitat, mainly dependent on a year's weather conditions. It was shown that data from official weather stations do not represent the microclimate, especially relative humidity, ticks are actually exposed to in their natural habitat. These findings already explain why single factors, explaining the suitability of a ticks' habitat are rare, and results are hardly transferable between different regions. The newly invented method of calculating development indices for ticks, on the basis of official weather data, offers researchers the opportunity to predict *I. ricinus*' distribution boundaries in the future. The first high-resolved map of *Ixodes* *ricinus'* density distribution was compiled for Baden-Württemberg (published in Boehnke et al. 2015). Therefore a generalized linear model (GLM) approach was used.

In ecological tick science, concepts that simplify the high degree of complexity and help to combine single findings are largely missing. Two ecological concepts were adapted to these purposes: the “concept of limiting factors” and the “concept of scales”. These concepts were combined to the tick specific concept of “essential and key factors” which offers a deeper understanding of processes behind tick patterns and can be used to easily structure knowledge of tick ecology in the future. A superordinate conceptual model illustrates the relationship between tick densities and environmental key factors at different spatial scales. Furthermore, a guideline for the combination of environmental and tick data for ecological tick research at different spatial scales is introduced.

Findings of this study point out how important it is to implement specific standards for European, ecological tick research in the future. Close attention should be paid on the detailed examination of the ticks' microhabitat and its influence on the ticks' physiologically-essential factors in respect to the spatial embedding of the habitat.

ZUSAMMENFASSUNG

Die Zeckenart *Ixodes ricinus* ist derzeit in Mitteleuropa der wichtigste Krankheitsüberträger. In Baden-Württemberg ist sie insbesondere durch die Übertragung der Frühsommer-Meningoenzephalitis und Lyme-Borreliose auf den Menschen bekannt. Das Risiko für eine zeckenübertragene Krankheit steigt mit der Wahrscheinlichkeit eines Zeckenbisses und damit mit der Anzahl von Zecken. Dennoch ist es bis heute nicht möglich, anhand der Eignung eines Habitats, also basierend auf relevanten Umweltfaktoren, die Abundanz von Zecken in einem bestimmten Habitat abzuschätzen. Daher war es das Ziel dieser Studie eine Reihe von geeigneten Methoden, Schlüsselfaktoren und Konzepten am Beispiel von Baden-Württemberg zu entwickeln, die es ermöglichen sollen die Eignung eines Habitats für *Ixodes ricinus* Zecken zu bestimmen.

Von Herbst 2012 bis Herbst 2015 wurden Baden-Württemberg weit an 25 Standorten insgesamt 22.697 *I. ricinus* Zecken gesammelt, kontinuierlich eine Vielzahl an mikroklimatischen Daten gemessen, sowie Daten zu Vegetation und Boden erhoben. Es wurden vielfältige Ansätze verfolgt, um diesen umfassenden Datensatz auszuwerten. Dabei wurden die in der Zeckenforschung üblichen Methoden verwendet. Teilweise wurden Methoden anderer Fachrichtungen angepasst. Wieder andere wurden hinsichtlich der Physiologie der Zecke für bestimmte Fragestellungen speziell entwickelt. Zusätzlich wurde das Habitat „Wald“ in die für die Zeckenbedürfnisse wesentlichen ökologischen Prozesse und Strukturen zerlegt.

Dadurch wurde eine Vielzahl neuer Erkenntnisse gewonnen. Beispielsweise zeigte sich, dass die Humusschicht ein Schlüsselfaktor für die Wasserversorgung der Zecken ist. Der Faktor „Humus“ beeinflusst die Zeckendichte eines Habitats, aber auch deren Entwicklungsdauer und sehr wahrscheinlich sogar den Zeitpunkt ihres Aktivitätsbeginns im Frühjahr. Der Effekt, den ein bestimmter Umweltfaktor auf die Zeckenabundanz ausübt, variierte deutlich von Jahr zu Jahr und von Standort zu Standort, vermutlich aufgrund der Jahreswitterung. Es konnte gezeigt werden, dass Daten offizieller Wetterstationen, insbesondere die der relativen Luftfeuchte, die tatsächlichen mikroklimatischen Bedingungen im natürlichen Zeckenhabitat nicht abbilden können. Diese Erkenntnisse erklären bereits, warum bisher so wenig allgemeingültige Faktoren zur Abschätzung der Habitateignung für Zecken identifiziert wurden. Die neu entwickelte Methode, Maßzahlen der Zeckenentwicklung auf Basis von offiziellen Wetterdaten zu berechnen, ermöglicht es Zeckenforschern die Verbreitungsgrenze von *I.ricinus* in Zukunft zu berechnen.

Erstmalig wurde eine hochaufgelöste Karte der Zeckendichte-Verbreitung für Baden-Württemberg erstellt (veröffentlicht in: Boehnke et al. 2015). Basis dafür ist ein generalisiertes lineares Modell (GLM).

In der ökologischen Zeckenforschung fehlt es bisher weitestgehend an Konzepten, mit Hilfe derer die hohe Komplexität reduziert und einzelne Erkenntnisse zusammengeführt werden können. Zwei ökologische Konzepte wurden daraufhin angepasst: das Konzept des limitierenden Faktors und das des Skalenniveaus. Diese wurden in einem zeckenspezifischen Gesamtkonzept, dem Konzept der „Basis – und Schlüsselfaktoren“, vereint. Das Gesamtkonzept ermöglicht ein tieferes Verständnis für die Prozesse, die eine Zeckenpopulation im Hintergrund beeinflussen, und kann in Zukunft dafür genutzt werden das Wissen zur Zeckenökologie in einfacher Weise zu strukturieren. Der Zusammenhang von Zeckendichte und Umwelt-Schlüsselfaktoren in Relation des Skalenniveaus wird in einem übergeordneten, konzeptionellen Modell veranschaulicht. Darüber hinaus wird ein Leitfaden für die ökologische Zeckenforschung zur Kombination von Zecken- und Umweltdaten in Abhängigkeit ihres Skalenniveaus vorgestellt.

Die Erkenntnisse dieser Studie betonen wie wichtig eine Implementierung von spezifischen Standards für die zukünftige, ökologische Zeckenforschung in Europa ist. Dabei sollte besondere Aufmerksamkeit auf die Untersuchung des Mikrohabitats und seinen Einfluss auf die physiologisch bedingten Grundbedürfnisse der Zecken gelegt werden, unter Berücksichtigung der räumlichen Einbettung des Habitats.

PUBLICATIONS

Parts of this thesis have already been published:

BRUGGER, K., BOEHNKE, D., PETNEY, T., DOBLER, G., PFEFFER, M., SILAGHI, C., SCHAUB, G.A., PINIOR, B., DAUTEL, H., KAHL, O., PFISTER, K., SÜSS, J. ,RUBEL, F. 2016. A Density Map of the Tick-Borne Encephalitis and Lyme Borreliosis Vector Ixodes ricinus (Acari: Ixodidae) for Germany. *Journal of Medical Entomology*.

BOEHNKE, D., BRUGGER, K., PFÄFFLE, M., SEBASTIAN, P., NORRA, S., PETNEY, T., OEHME, R., LITTWIN, N., LEBL, K., RAITH, J., WALTER, M., GEBHARDT, R. ,RUBEL, F. 2015. Estimating Ixodes ricinus densities on the landscape scale. *International Journal of Health Geographics* 14:23.

PETNEY, T., PFÄFFLE, M., LITTWIN, N., NORRA, S., BÖHNKE, D., HOGEWIND, F., GEBHARDT, R., OEHME, R., SEBASTIAN, P., STEIDLE, J., KAHL, O., DAUTEL, H. 2015. Untersuchung der Ökologie von Zecken als Überträger von Krankheitserregern in Baden-Württemberg im Bezug auf Habitat, Landnutzung, Wirtstiere und Klima (Zwischenbericht). FADO, LUBW

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Last but not least, a warm and heartily "Thank you so much!!" goes to my family and friends, who were always there for me and accompanied me through all ups and downs. Thank you!!!

CONTENTS

1 INTRODUCTION

1.1 OVERVIEW

Nowadays *Ixodes ricinus* is certainly the most-common, most widely distributed and best studied European tick species. Their wide distribution is mainly due to their high adaptability to different environmental conditions, as well as their low host specificity. However, especially their low host specificity causes that this species acts as the main vector of diseases to humans and animals in Europe. In addition, newest findings also indicate that *I. ricinus* tick bites are associated with red meat allergies and those emerge even in Baden-Württemberg (Hamsten et al. 2013, Fischer & Biedermann 2016). In this context information about the presence of the species and the dependence of their population density of environmental factors are relevant to assess to the potential risk of a tick bite and associated disease transmission.

Interestingly, we are still not able to predict, for example, the altitude-dependent distribution limit of the species in a mountain range, or to approximate the tick abundance of a specific habitat on the basis of a set of environmental factors in remote regions. There are two interlinked causes for that: 1) the identification of possible biotic and abiotic factors influencing ticks abundance in the field is hampered by the multiplicity, complex interdependency and temporal variability of these factors and 2) tick abundances reflect the suitability of local conditions, which can restrict the transferability of results to other regions (for example: Merler et al. 1996, Burri et al. 2007, Martello et al. 2014). In addition, the acquisition of tick data and of potential environmental factors is not yet standardized, which can restrict the comparability of results and, in some cases, used climatic data, e.g. obtained from official weather stations, seem even inappropriate for the research question (reviewed in Estrada-Peña et al. 2013).

Therefore it is quite unclear, which and to which extent environmental factors are locally relevant for *Ixodes ricinus* ticks and, as a consequence of this, what kind of spatio-temporal pattern of tick abundances are to be expected in a specific region.

The study area Baden-Württemberg is located in the center of the distribution area of the tick species *Ixodes ricinus* and is characterized by a high risk of human infections with FSME and Borrelia species. The aim of this study was to create a set of useful methods, key factors and concepts, which help to identify the suitability of a habitat in its regional setting for *Ixodes* *ricinus* ticks, on the example of Baden-Württemberg, as an elementary step to identify areas at high risk of a tick bite in the future.

Limits regarding tick research are described in the following. The habitat suitability of a tick can fundamentally be described using the two governing factors food resource (availability of suitable host for the blood meal) and physical resource (vegetation and associated microclimate) (Wilson 1998). In the scope of this work the focus is on the physical resources, especially the (micro) climatic factors temperature and relative humidity and parameters influencing the latter. Only forest habitats were examined within the high ecological variety of the study region since these are expected to be the main habitat for *I.ricinus* in Baden-Württemberg. Further, my analysis and explanations are strongly orientated on the needs of this research field. Therefore, after giving a detailed summary of important ecological background knowledge in the first chapter, complexity is later mostly reduced to elementary elements that are commonly used in ecological tick research. There is a need for this limitation because the impact of environmental factors on tick densities is an extensive complex area.

The thesis was partly generated within the interdisciplinary research project “ZUP – Ökologie von Zecken als Überträger von Krankheiten in Baden-Württemberg” financed by the Department of Environment Baden-Württemberg in the BWPLUS program, financed by the Graduate School for Climate and Environment (GRACE) and the Institute of Geography and Geoecology of the Karlsruher Institute of Technology (KIT).

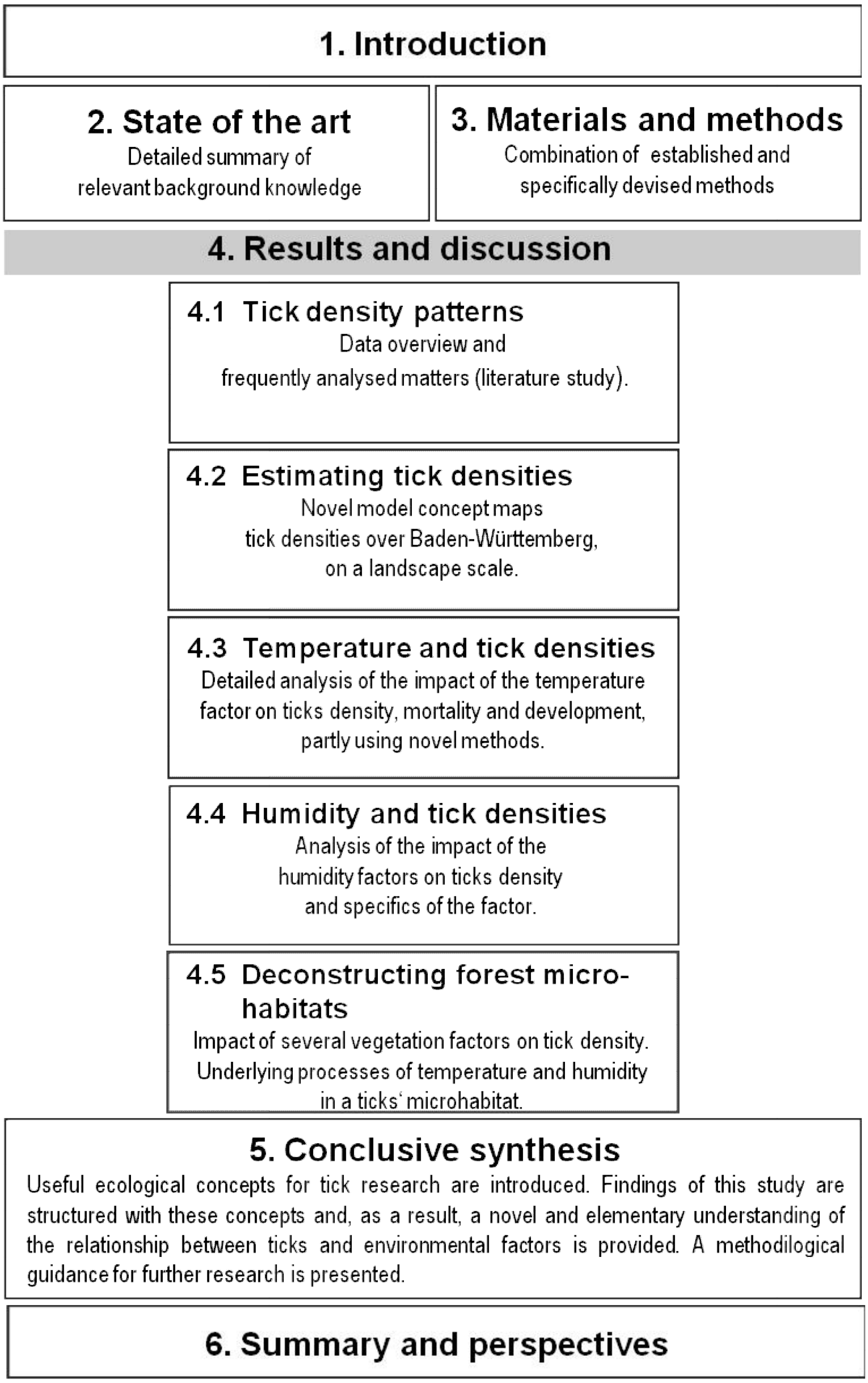
1.2 STRUCTURE OF THE THESIS

The structure of this thesis is as follows:

Chapter 2 gives an overview of the current state of research concerning the studied species *Ixodes ricinus*, ecological and microclimatological aspects of forest habitats and the study area Baden-Württemberg.

Chapter 3 contains all materials and methods used, including the choice of study sites, microclimatic measurement concepts, the vegetation survey and tick sampling as well as descriptions of developed and adopted data processing techniques and of the applied statistical methods.

Chapter 4 is divided into six sub-chapters which focus on specific subject areas. First, the general climatic and tick-related situation in Baden-Württemberg is described.



1. Introduction

2. State of the art
Detailed summary of
relevant background knowledge

3. Materials and methods
Combination of established and
specifically devised methods

4. Results and discussion

4.1 Tick density patterns
Data overview and
frequently analysed matters (literature study).

4.2 Estimating tick densities
Novel model concept maps
tick densities over Baden-Württemberg,
on a landscape scale.

4.3 Temperature and tick densities
Detailed analysis of the impact of the temperature
factor on ticks density, mortality and development,
partly using novel methods.

4.4 Humidity and tick densities
Analysis of the impact of the
humidity factors on ticks density
and specifics of the factor.

4.5 Deconstructing forest micro-habitats
Impact of several vegetation factors on tick density.
Underlying processes of temperature and humidity
in a ticks' microhabitat.

5. Conclusive synthesis
Useful ecological concepts for tick research are introduced. Findings of this study are structured with these concepts and, as a result, a novel and elementary understanding of the relationship between ticks and environmental factors is provided. A methodilogical guidance for further research is presented.

6. Summary and perspectives

The second chapter contains a new approach to estimate *I. ricinus* nymphal densities on a landscape scale. Thirdly, findings concerning the impact of the factor temperature on tick densities are presented.

The fourth part explains why relative humidity is not a factor that can be examined at the same level as temperature before. Thus, in the fifth part, new insights in the microhabitat of ticks are presented, relevant aspects of several environmental parameters are pointed out and underlying processes concerning temperature and relative humidity patterns are explained.

Chapter 5 gives a conclusive synthesis of all sub-chapters and introduces useful theoretical concepts for tick research, which reduce complexity and offer new ways to structure tick-environment related knowledge.

Chapter 6 contains a detailed summary of the most relevant findings of each chapter and conclusions and perspectives on future work will be given.

2 STATE OF THE ART

2.1 IXODES RICINUS

2.1.1 INTRODUCTION

Nowadays, one of the most comprehensive works on ticks is the “Biology of Ticks” (Sonenshine & Roe 2014). As mentioned there, the oldest ticks were found preserved in fossil amber of about 50 million years of age. As a species, ticks were described for the first time by Linnaeus in his “Systema Naturae” from 1758. Ticks taxonomically belong to the phylum of *Arthropoda*, the class of *Arachnida* (spider-like animals) and are grouped together with mites as the subclass *Acaria.* In Europe*,* two families occur: Ixodidae (hard ticks) and Argasidae (soft ticks) (Mehlhorn & Piekarski 2002). Hard ticks differ from soft ticks in the presence of a hard shield, i.e. a Scutum. *Ixodes ricinus* belongs to the family of hard ticks (Ixodidae), which is the largest of the families with 14 genera, approximately 700 (sub) species (Petney et al. 2012) and has the greatest medical and economic significance (Heyman et al. 2010). Intensive research on the species *Ixodes ricinus* was at first carried out in Britain (Macleod 1932, Milne 1943, Lees 1947) since the so called “sheep-tick” had been recognized as a harmful disease transmitter on sheep and cattle. Nowadays, it is certainly the most-common, most widely distributed and best studied European tick species (Petney et al. 2012)

In Europe, *I. ricinus* is the main vector of two serious diseases for humans. It transmits *Borrelia burgdorferi* bacteria causing Lyme borreliosis and the main vector and reservoir for the *tick-borne encephalitis virus* (causing the so called FSME). In addition, this species is the only known vector causing human granulocytic anaplasmosis, vector of some Rickettsial subspecies with indifferent disease pattern and vector of several types of protozoa causing the disease Babesiosis in lifestock, dogs, wild animals and humans (Heyman et al. 2010). Of those, *Borrelia* bacteria are the most prevalent tick-borne pathogens in humans in Europe. The incidence of the transmitted disease Lyme borreliosis have been found to increase in many countries over the last decade (Rizzoli et al. 2011, Medlock et al. 2013).

A tick bite always bears the risk of catching a serious disease for humans as well as for animals. Within the scope of this work, the focus lies on the chance to be bitten by a tick, not on single pathogens or on diseases. Since the probability to serve as a tick-host in a specific habitat naturally increases along with the numbers of ticks, the analysis focuses on factors influencing *I. ricinus* survival and mortality in a habitat and on a large-scaled pattern of its density distribution.

2.1.2 DISTRIBUTION AND ABUNDANCE

Ixodes ricinus occurs throughout the European continent up to the Ural mountains in the East, Great Britain in the West, Sweden in the North and North Africa in the South (Randolph et al. 2002). In central Europe, the tick occurs from the lowlands to the mountains (Krippel & Nosek 1978). Distribution boundaries have been found up to altitudes of 1100 m a.s.l. in the Czech Republic (Materna et al. 2008) and up to 1650 m a.s.l. in Italy (Martello et al. 2014). Due to global warming, an expansion of the tick further north (Lindgren et al. 2000, Jaenson & Lindgren 2011) and the occurrence of *I. ricinus* ticks at higher altitudes (Daniel et al. 2003) combined with an increase of tick abundances have been documented over the last decades.

Georeferenced locations of several tick species in Germany are given in Figure 2.1. (Rubel et al. 2014). Figure 2.2 provides a large-scaled map of the potential distribution of *I. ricinus* in the western Palearctic, based on 4,000 places where ticks occurred and the probability that ticks are able to develop and survive, which is modeled by temperature and vegetation factors (Estrada-Peña et al. 2012). It can be seen that Germany is located in the center of distribution and provides usually suitable conditions for this species.

As a general rule, tick abundances reduce with elevating altitudes (Jouda et al. 2004, Medlock et al. 2013). This was found to be related to a lesser percentage of successfully hatching eggs as well as a lesser chance for larvae and nymphs to find a host and to develop to the next stage within one season along with growing altitude (Danielová et al. 2006, Materna et al. 2008).

Ticks are not able to actively leave an unsuitable habitat. It was found that the largest distance larvae of *I. ricinus* were able to move in a day was 2.5 m and that in a month the spread from a nest was 8.5 m^2 at maximum (Daniel & Dusbabek 1994). At a local scale, ticks change habitats only via their hosts and it depends mainly on the host species how far and where ticks are transported, which can be crucial for further surviving. In the new microhabitat, ticks will search for shelter near the ground to stabalize their body's water balance and move vertically along the vegetation when actively questing for the next host (Randolph 2004, Sonenshine & Roe 2014).

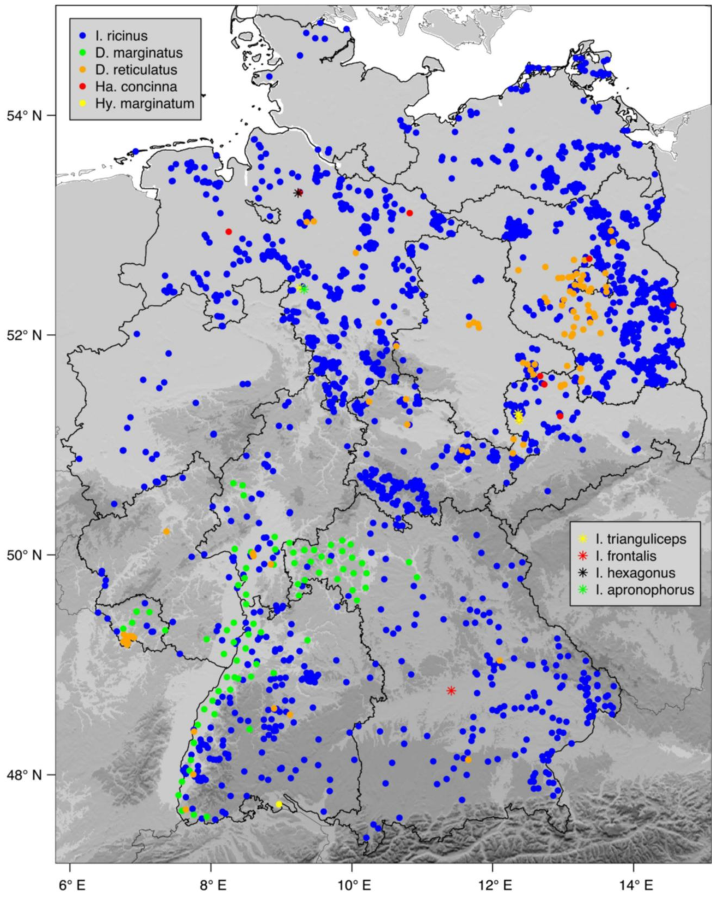


Figure 2.1: Georeferenced ixodid tick locations in Germany with *I. ricinus* labeled as blue dots (Rubel et al. 2014).

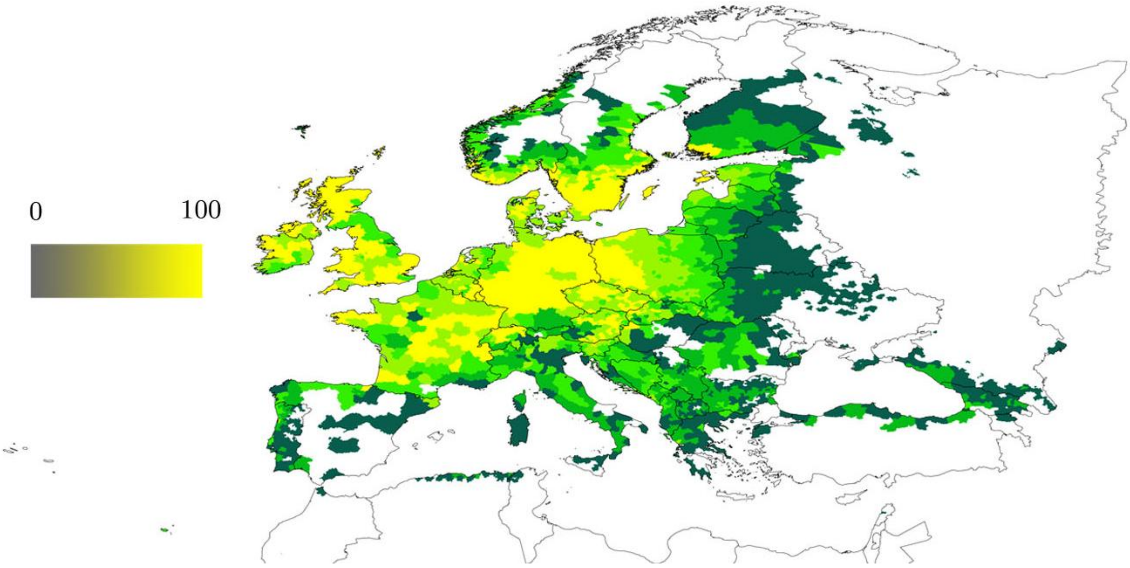


Figure 2.2: Predicted climatic suitability (yellow = very suitable to dark green = less suitable) for development and surviva of the tick *Ixodes ricinus* in the western Paleartic (Estrada-Peña et al. 2012).

Changes in tick abundances and cases of tick-borne diseases were also attributed to changes in anthropogenic intervention in habitat characteristics, e.g. fragmentation and tree species composition, and consequential changes in microclimatic and host associated conditions (Randolph 2001, Sumilo et al. 2008, Kiffner et al. 2010, Lauterbach et al. 2013, Korotkov et al. 2015). The abundance of ticks and infection risk for humans have been found to increase along with the fragmentation of the landscape.

Considering all the latter information the following becomes obvious: Each region provides a more or less unique combination of climatic and microclimatic conditions, habitat types, host communities and anthropogenic interventions, which all together shape the abundance and activity of the local tick population. Most surveys can provide an interesting insight into the rules of a specific habitat, and could be generally transferred to other habitats' but too little has been done to search for reproducible factors and to evolve a mechanistic understanding that is needed to integrate results in a larger context.

Consequently, this thesis focuses on 1) the integration of all information to find relevant structures and transferable rules behind the spatial heterogeneity of questing tick densities in Baden-Württemberg and beyond (Chapter 4.3 and 5) and 2) the mechanistic understanding and concrete description of the microclimate within the ticks' habitat with a high tempo-spatial resolution (Chapter 4.4 and 4.5).

2.1.3 ECOLOGY OF IXODES RICINUS

Ixodes ricinus ticks are ectoparasites with three living stages (larvae, nymphs, adults), each requiring a bloodmeal from a suitable host to develop to the next stage or for reproduction. Figure 2.3 and Figure 2.4 illustrate these living stages, their development and activity throughout the year as well as their most associated hosts. The ticks attach to hosts via the vegetation, where they wait for suitable hosts to come by (Sonenshine & Roe 2014). Most mammals, reptiles, birds, lifestock and humans can serve as hosts and are therefore at risk of contracting a disease (Oliver 1989). Each life stage has a host size preference, which is connected to the height of active questing in the vegetation. Larvae prefer small mammals and birds when questing up to 10 cm above the ground. Nymphs prefer small to medium sized mammals (including humans) questing up to 1 m and adults are most common on large animals such as deer and lifestock (e.g., sheep, cattle) questing up to 1.5 m (Krippel & Nosek 1978).

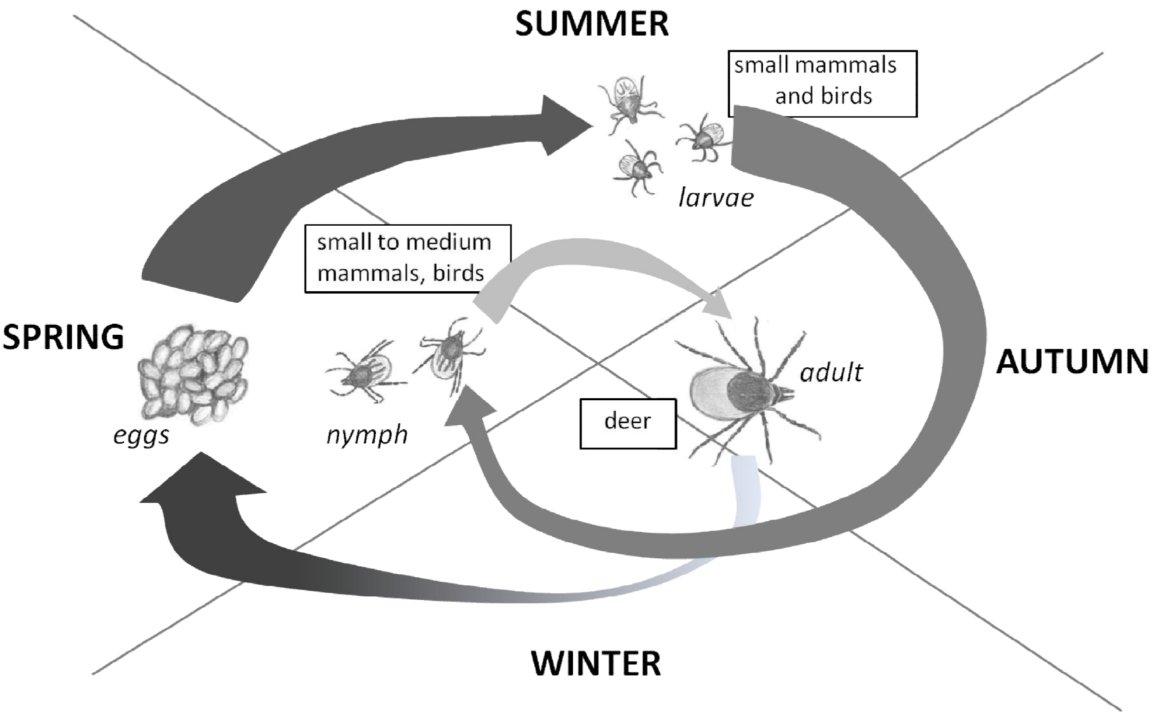


Figure 2.3: Seasonal activity pattern of *I. ricinus* life stages with principal hosts and implied losses of tick individuals from stage to stage. Author's own drawing, following van Buskirk & Ostfeld (1995).

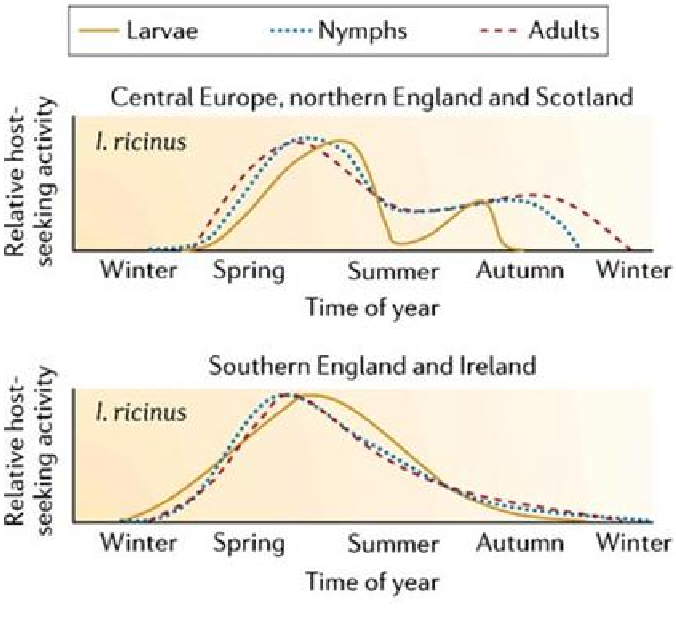


Figure 2.4: Typical seasonal activity of different *I. ricinus* life stages in Europe. The top graph shows bimodal, the lower graph monomodal pattern (modified, from Kurtenbach et al. 2006).

The seasonal behaviour of ticks is not only a matter of short-time weather conditions, as described in the next chapter, but rather an evolutionary adaptation to the regional climate and activity pattern of relevant hosts (Dautel 2010). The activity throughout the year is also called tick **phenology**, results from field studies indicating that mainly two behavioral variations occur: **monomodal** and **bimodal**, illustrated in Figure 2.4. Kurtenbach et al. (2006) differentiated ticks phenology large-scaled, i.e. by regions. However, results from southern Germany indicate that these pattern also coexist on a much smaller scale (Schulz et al. 2014) and Kurtenbach et al. (2006) stated that these pattern can also fluctuate strongly between years at the same location. In addition, the different life stages have been found to differ in their phenology: Nymphs and adult ticks usually show a bimodal pattern with an activity peak in spring and another in autumn, while larvae occur mainly in summer, showing a monomodal activity pattern (Estrada-Peña et al. 2004, Kurtenbach et al. 2006).

These patterns depend mainly on environmentally induced **diapause**, a "neurohormonally mediated dynamic state of low metabolic activity” (Tauber et al. 1986), that allows ticks to seasonally synchronize their activity with suitable time periods by a delay in the onset of development (morphogenetic diapause) or delayed host-seeking activity after hatching or moulting (behavioral diapause) (Belozerov 1982, Ogden et al. 2004, Dautel 2010). The day length, i.e. the photoperiod, has been described as the major trigger for the onset of diapause. The threshold of this onset depends also on temperature, with a lower threshold at higher temperatures (Belozerov 1982) – indicating an adaption to conditions in summer. As a result, the onset of the ticks’ development is influenced by the time of feeding. Randolph et al. (2002) found that ticks develop immediately to the next stage within a season if they feed before July, but delay processes to springtime in the following year if they feed later. Olaf Kahl explains this behavior by a very high mortality for ticks that haven`t completed their development until winter. Thus, ticks start development only if the remaining time span in the season allows the completion of the whole process until temperatures get to low (speech at the 3rd Zeckenkongress 2016, Hohenheim).

The bloodmeal is the only source of energy available to the tick to develop to the next stage and for their subsequent active questing phase to find the next host. Experiments with fat contents of nymphs indicate that they have a maximum energy storage for at least four month if conditions are perfect. If ticks are, e.g., forced to move and to absorb water actively due to drought stress, the energy level sinks faster and the lifetime shortens (Randolph & Storey 1999). However, laboratory tests by MacLeod (1932) showed that unfed larvae survived for two years and some of them were actually able to feed after 15 month, while some of the nymphs were able to feed after 13 months – indicating that nymphs are able to survive longer than four month as stated by Randolph & Storey (1999).

However, not only the energy storage of a tick influences its behavior and survival prospect. Since ticks are not able to change their habitat actively, they strongly depend on sufficient environmental conditions at the place where they drop off their hosts. The next chapter gives an overview of environmental factors relevant for this thesis and their influence on *I. ricinus* living processes.

2.1.4 IMPACT OF ENVIRONMENTAL FACTORS

Ticks spend 98 to 99% of their lifetime off-host, i.e. within the microenvironment near the ground surface (Kahl 1989), so environmental factors are expected to have a major impact on the ticks' development, behaviour and mortality. Figure 2.5 illustrates different phases each tick has to go through after hatching (larvae) or moulting (nymphs, adults) and denotes influencing environmental factors for each phase.

After hatching or moulting, the cuticle is soft and needs to harden before a tick gains its full protection ability - a procedure that takes several days to weeks. In this time, ticks are very sensitive to desiccation and depend strongly on sufficient humidity in their microhabitat, thus showing no host seeking behaviour (behavioural diapause) (Dautel 2010). Afterwards, a tick needs to find the next host for further development and starts questing on the vegetation. The temperature and humidity conditions ticks are now exposed to result from a combination of season, daytime, weather and vegetation characteristics. If conditions are too dry, ticks will lose water and are subsequently forced to move to moister areas near the ground surface and to absorb water actively – an energy consuming process (see Figure 2.6). Therefore, some researchers expect *I. ricinus* to quest mainly at nighttime where the relative humidity is higher compared to daytime (Randolph & Storey 1999). The chance of finding an adequate host (illustrated in Figure 2.3) increases with the abundance of this host, which is mainly a result of food supply and habitat suitability, i.e. vegetation structure and species composition. After successfully feeding on a host, ticks drop off, search for shelter in the litter layer (forest), under plant remnants (open field) or within the upper soil layer and develop to the next stage.

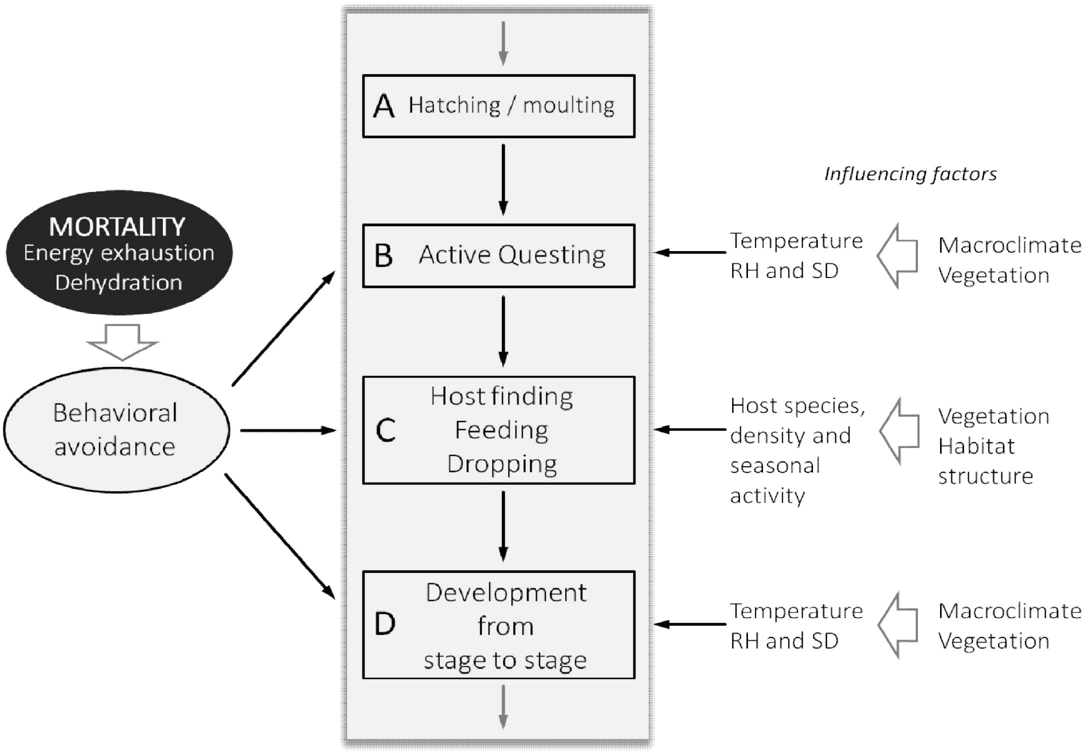


Figure 2.5. Impact of selected environmental factors in different phases of the ticks' life cycle (based on (Randolph 2004), largely modified).

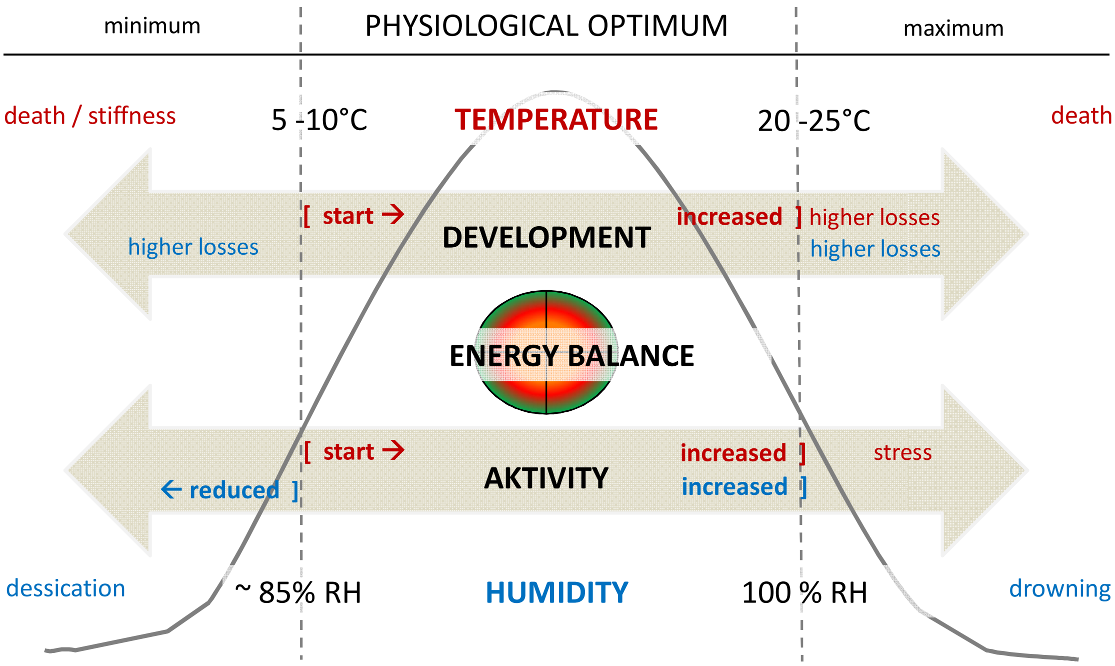


Figure 2.6: Impact of temperature and humidity on ticks' living processes.

Figure 2.6 images how the main drivers temperature and humidity affect ticks living processes on the basis of its physiology. Temperature and humidity related responses of ticks often happen simultaneously or determine one another. However, in the following, both climatic factors and their influence on ticks are presented seperately to simplify the connection to the particular results and discussion chapter.

Since *I.ricinus* is a poikilothermal species, its living processes are mainly restricted by thermal conditions (Dautel 2010). In detail, temperature is the fundamental factor that

- limits start and end of seasonal tick activity in a year
- limits its spatial distribution to the north and higher altitudes
- is responsible for the time span needed for development
- strongly influences mortality, especially by extreme events in winter and summer

To give an overview: There is a lower limit that prohibits the ticks' mobility and development if temperatures fall below it. Above that limit, the ticks' metabolism, locomotor and questing activity as well as development speed increases along with rising temperature, which also characterizes the ticks' normal and optimum temperature range. If temperatures get too high, it was observed that ticks show a stress behaviour at first and afterwards an escape behaviour to avoid damaging conditions (Sixl & Nosek 1971).

The temperature factor influences tick behaviour seasonally and diurnally. After winter, weekly mean daily maximum temperatures of 7°C may be a threshold for the onset of questing activity of nymphs and adults in temperate regions (Perret et al. 2000, Randolph 2004, Randolph et al. 2008). When active, temperature preferences of the life stages largely coincide: Nymphs showed a normal activity at 16 to 20°C, larvae at 10 to 22°C and adults at 18 to 25°C under laboratory conditions (Sixl & Nosek 1971). Related to macroclimatic conditions, *Ixodes ricinus* populations in Germany are active mainly between March and October.

The ticks' development is also restricted to this warm period, since related processes are largely temperature driven (Dautel 2010). Campbell (1948) determined the relationship between temperature and development progress for each tick stage with laboratory experiments. Results are expressed as equations, which calculate a tick's daily development progress under the given temperature. He found that, after development started at about 8°C, the development speed increases non-linearly (stronger) with increasing temperature. In addition, it seems that permanently cool conditions have a negative influence on developmental success and thus survival of individual ticks in nature. This conclusion is based on results by Materna et al. (2008) who found that the percentage of successfully hatching ticks decreases strongly with increasing altitude where temperatures get cooler.

Ticks overwinter in a status called winter-quiescence. They developed a variety of physiological and behavioral strategies to maximise their survival prospects in winter. For example, ticks assemble a physiological protection against cold, burrow themselves into the leaf litter and upper soil layer, if possible, and thus benefit from a snow cover that reduces temperature changes and extreme temperature events (reviewed in Dautel 2010). However, mortality has been found to increase if ticks are exposed to extreme cold temperatures (< -16°C) and direct contact with ice, long periods of freezing temperatures (Dautel & Knülle 1997, Dantas-Torres et al. 2013) or frequent diurnal temperature variations (Herrmann & Gern 2013). In addition, Dautel (2010) stated that tick mortality caused by temperature drops is higher in springtime after a warming phase when ticks have lost their physiological protection against cold compared to those in winter under full protection. However, reports of questing ticks in winter were more frequent in the last years which is attributed to milder temperatures due to global warming (Dautel et al. 2008, Schulz et al. 2014).

The humidity supply in a given habitat is responsible for tick survival and their duration of activity, since it influences restricts the ability to maintain a sufficient body water level. In a subsaturated atmosphere, ticks lose water, e.g., by discharging faeces and urine, by gas exchange, and via the cuticle (Kahl 1989). The saturation deficit is a measure of the drying power of the atmosphere and as such largely responsible for the rate of body water losses of individual ticks in a given situation, which gradually leads to a leathal dessication level (Perret et al. 2000). Ticks are in general able to restore their body water balance by active water vapour uptake when the relative humidity (RH – see also List of Abbreviations, Chapter 7) surpasses a certain threshold. This so-called critical equilibrium humidity was found to be about 85% RH in laboratory experiments (Kahl & Knülle 1988). The process of active water absorption needs energy and thus leads to a faster consumption of a ticks' fat storage, which is restricted to some weeks or even days when ticks are not in quiescence but active (Lees & Milne 1951). As a result, ticks were found to move to microhabitats of higher relative humidity after they were exposed to dry conditions (Lees 1947).

Temperature can restrict the ticks' abundance in a habitat, especially in areas near their distribution boundary like areas of high altitude and northward or Mediterranean regions.

The first question to be answered is what kind of thermal (distribution) boundaries are represented in Baden-Württemberg and where these are. Sites will be characterized by relevant abiotic thermal parameters on the one hand and tick development dependent parameters on the other hand. Next, the content of the results are integrated into a larger ecological context and interdependences with other factors like vegetation or geomorphological aspects are discussed. On this basis, thermally favourable and disfavourable regions will be defined.

2.2 MICROCLIMATE IN FOREST HABITATS

The climate within a forest has a specific characteristic compared to the climate at the free atmosphere. A scientific report recently stated that open field data from official weather stations lack information on how climate change influences subcanopy temperatures (Frenne & Verheyen 2016), which is certainly also transferable to moisture conditions. Since ticks are very sensitive to the impact of climatic conditions (see Chapter 2.1.4), this chapter aims to give a detailed insight into the influence of specific forest characteristics on its subcanopy microclimate.

2.2.1 GENERAL ASPECTS AND DEFINITIONS

First, the term "microclimate" has to be set in context. Following Geiger (2009) and Linacre (1992), a definition of "the abstract concept" of climate could be: "Climate is the synthesis of atmospheric conditions characteristic of a particular place in the long term. It is expressed by means of averages of the various elements of weather, and also the probabilities of other conditions, including extreme events."

Geiger et al. (2009) classify four types of climatic scales used to explain climatic phenomena. There are different attempts to quantify the horizontal, vertical and temporal ranges of these scales for a general usage although it is well known that these ranges differ depending on local and regional settings.

Accordingly, for this work, scales and ranges are defined in a qualitative rather than a quantitative way. The four types of climatic scales are as followed:

- The **macroclimate** represents the climatic effects of atmospheric circulation on a continental scale (Geiger et al. 2009)
- The **mesoclimate** represents the regional scale, where topographical aspects, e.g. relief and altitude, modify the macroclimate.

  This includes, e.g. mountain-plain circulations, reduced precipitation rates in regions behind a mountain lee (for example at the southern part of the *Schwäbische Alb* (Borcherdt 1983) or long-term pools of cold air in valley basins (*Baar* region (Kappas 2003)

The following scales are defined regarding the requirements for this thesis and are therefore more distinct compared to the definitions made by (Geiger et al. 2009).

- The **local climate** comprises effects depending on relief and vegetation, which cause specific local climatic conditions.

  A north-facing versus a south-facing slope for instance, or a forest stand versus the neighboring open field. These local aspects change conditions compared to the surroundings as well as determine the microclimate conditions within (Suggitt et al. 2011).
- The **microclimate** represents the smallest scale and considers the variability of conditions to be found in a specific habitat domain.

  For example the litter zone versus the herb zone versus the canopy of a broad-leaved forest stand, or the conditions within the vertical range of a grass patch.

Figure 2.7 and 2.8 provide an overview of the interactions of climatic variables, habitat types and the soil domain. For the scope of this work, the focus lies on the microclimate within forest stands, processes and patterns behind and its significance for the species *Ixodes ricinus*. As described in detail in the last chapter, the main habitat of this species are forests, which makes microclimatic pattern and processes in forest habitats a very important source of knowledge, since ticks are highly dependent on temperature and moisture conditions near the forest due their limited, active mobility.

The next part introduces some general aspects of climatic conditions in forest stands:

The forest **canopy** highly modifies external factors. At daytime, the canopy reduces the incoming shortwave radiation by absorption and thereby slows the heating of the underlying layer. At nighttime, it reduces the amount of outgoing longwave radiation (effective emissivity) and thus reduces the cooling of the forest stand compared to the open field. As an example, a frost occurrence damaged nearly 75% of white spruce seedlings on a wide clearcut but only 2% of seedling sheltered by a close canopy (Groot & Carlson 1996).

Figure 2.8 gives an overview of these radiation-dependent processes, which can be summarized: With every insulating layer, the amount of incoming radiation, heat and light decreases – hence, the climate near the ground depends highly on the structure of the forest canopy (Otto 1994). Interception processes reduce the amount of **precipitation** that reaches the forest floor.

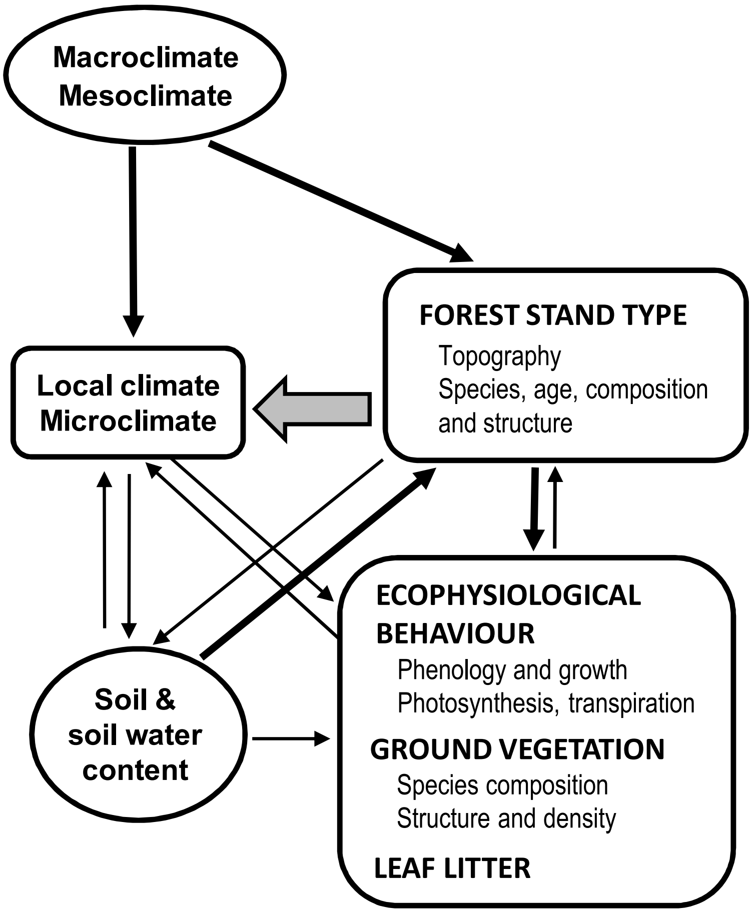


Figure 2.7: Interconnections between climate, soil conditions and forest stands (Aussenac 2000, modified).

The distribution of the precipitation on the ground depends on canopy structure (e.g. density) and tree shape. **Wind speed and turbulences** are reduced. The degree of reduction depends on the stand and canopy structure, foliage characteristics, tree age and species composition, occurrence and extension of clearings and the wind speed itself. Consequently, the **water storage** under the canopy is more balanced, since evaporation and transpiration processes are reduced and soil and air moisture remain higher (Otto 1994).

Concluding, the **forest stand highly modifies its internal microclimatic conditions**. Simultaneously, a forest stand is a part of the landscape it is located in and thus highly influenced by its local climatic setting. Topographic elements such as altitude and slope inclination can strongly modify the amount of incoming solar radiation in a forest stand and thus its thermal conditions (Lee 1978). The following chapter discusses in detail the effects of radiation (especially the incoming solar radiation) on forest stands along with underlying processes, and their importance for *Ixodes ricinus* ticks in regards to their physiology.

2.2.2 PROCESSES INFLUENCING AIR AND BODIES TEMPERATURE

Radiation is the primary factor driving microclimatic processes in a forest stand (Geiger et al. 2009).

The emitted solar radiation (“shortwave flux”) coming from the sun is, at first, modified within the atmosphere through several processes. Depending on the actual atmospheric conditions, a certain amount and composition of shortwave and longwave radiation (“global radiation”) reaches the surface directly or diffuses (“**incoming solar radiation**”) and will partly be absorbed, reflected or transmitted as a function of individual surface properties (Lee 1978). Since radiation is an electromagnetic form of energy, its absorption leads to a warming of the absorbing body. How much the temperature increases is a function of characteristic material properties such as its specific heat [J/(kg*K)] and thermal conductivity [SI: W/(m*K)] and of the penetration depth of the radiation (Blüthgen & Weischet 1980). Along with rising temperatures, the amount of emitted longwave (“terrestrial” or also “thermal”) radiation increases to balance the energy absorption of the body. Longwave radiation mainly occurs at nighttime, when absorbing bodies lose heat – a process called **nocturnal radiation** (Geiger et al. 2009).

The energy exchange at earth`s surface can be expressed by the following equation (Geiger 2009):

$$Q^* + Q_G + Q_H + Q_E = 0$$

Q^* = net radiation [W/m²]; the sum or net balance of incoming and outgoing radiation

Q_G = energy flow from the surface into the ground (-) and vice versa (+) [W/m²]

Q_H = energy flow from the surface to the air (-) and vice versa (+) [W/m²]

Q_E = latent heat; energy flow due to phase changing processes of water [W/m²]

Figure 2.8 illustrates the solar energy flow in a forest stand compared to the open field, the successive reduction of energy and connected processes within the forest as well as the dynamic energy exchange in the transition zone between forest and bordering open area. The canopy of a forest acts like a huge irregular surface where all the mentioned processes of the equation occur. A small part of the incoming radiation is reflected in the atmosphere, the remaining energy is transported from the canopy to subordinated layers by molecular and turbulent (so called "Eddy") diffusion processes (Geiger et al. 2009). The canopy is the first insulating layer retaining the incoming energy and therefore has a concise influence on intensity and quantity of the remaining radiation going into the forest.

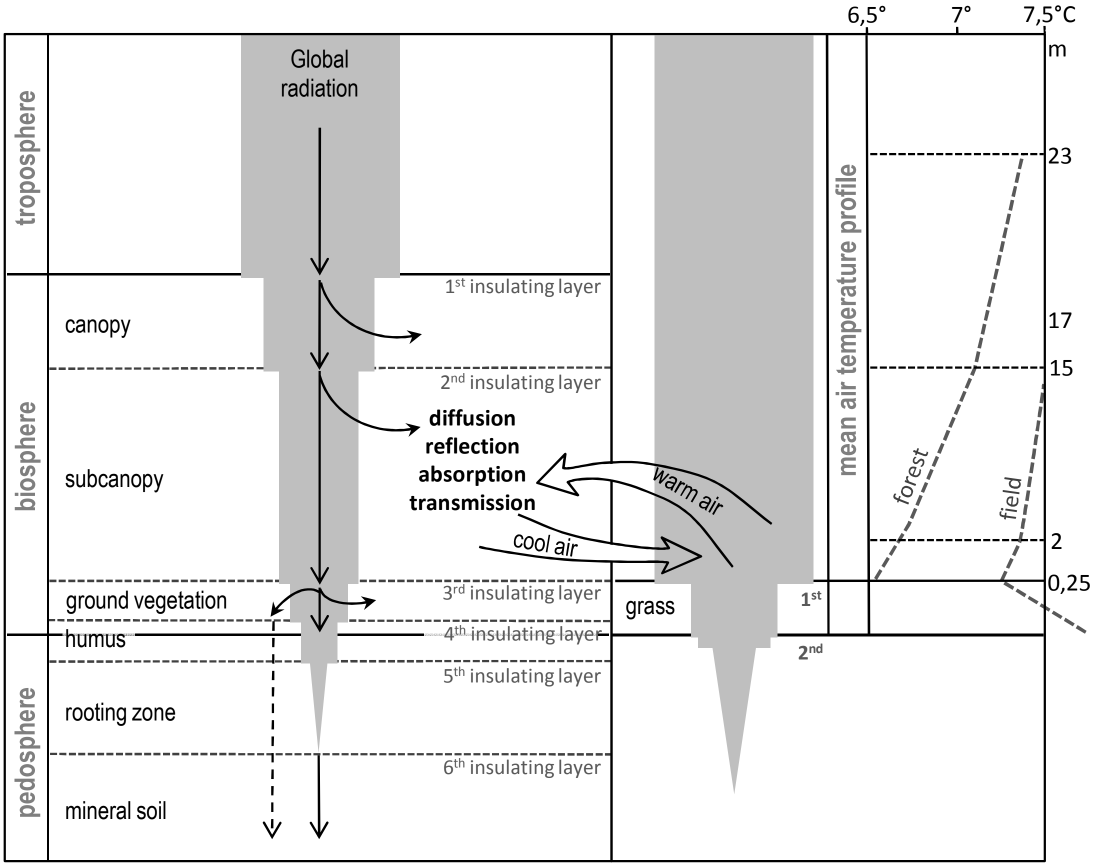


Figure 2.8: Energy input in and temperature profile of a forest (without shrub layer) and open field habitat at a sunny day; modified after (Otto 1994).

In each following insulating layer, the energy flow is reduced by absorption, reflection, diffusion and transmission. A dense canopy can retain a huge amount of incoming radiation, leaving only

1-5% of directly passing solar energy for the ground vegetation. The forest soil is even more shielded from radiation, depending on the depth and structure of the humus (Otto 1994).

The amount of solar radiation reaching the **forest floor**, where ticks live, depends on canopy structure, density and tree species composition due to transmission and absorption capacities of the leaves (Lee 1978). Another important factor is the season and, subsequently, the forest type. A **coniferous forest** (but for *Larix spp.*) has a constant canopy throughout the year with a maximum of incoming radiation in summer and a minimum in winter. (Otto 1994).

By contrast, in a **broad-leaved forest** the ground reaching radiation is highly variable during the year due to the foliage status and seasonal sun position and intensity. The incoming radiation has a minimum in winter, remains at a constant low level until spring, reaches its maximum in late spring and drops down in summer due to foliation processes. In autumn, along with defoliation, the amount of incoming energy rises slightly and then drops to the winter minimum (Hutchison & Matt 1977). In summertime, the incoming radiation depends on stand and canopy density and the vertical structure of the forest. Gaps in the canopy lead to **temporarily sunspots** on the ground causing a short-term, local warming (Otto 1994). In addition, in loose forest stands and especially at **clearings,** the amount of directly passing radiation is much higher compared to dense stands with a completely covering canopy.

The distribution of radiation at clearings depends mainly on its extent, the height of shadings trees at its borders and the angle of incidence of solar radiation and therefore varies seasonally and diurnally (Geiger et al. 1995).

In **springtime**, a major part of the incoming energy reach can the ground and lead to a warming of litter, humus and the soil beneath when trees are defoliated; a fact a lot of fast growing herbal plants are adapted to (e.g. *Anemone nemorosa*). To give an example: In an oak and beech forest in Belgium, 17/12% (foliated/non-foliated) of the incoming radiation was reflected, 76/52% absorbed and 8/36% was transmitted in fully foliated compared to non-foliated status. In contrast, temperature conditions in a coniferous forest are highly reduced due to its full foliage (Otto 1994).

Spring is also the **main period for tick activity and development** – with both processes strongly dependent on temperature (see Chapter 2.1.4). Since ticks overwinter in the upper soil and litter layer, a warming of these areas support activity start and physiological processes. Thus, conditions should vary distinctively for ticks in springtime between foliated (= coniferous) and defoliated (= deciduous) forests and, in addition, between dense and loose stands. How these terms influence *I. ricinus* ticks is one of the questions to be answered in this thesis (Chapter 4.5.2).

The warming through solar energy absorption is just one form of temperature transport inside a forest. Following the second law of thermodynamics, the temperature exchange (called sensible heat flux) directs from warmer towards lower temperatures until the difference is equalized (Lee 1978). As an example: When a temporarily sunspot causes local warming at the leaf litter surface, the heat flux towards litter and soil below and the atmosphere above (Figure 2.9).

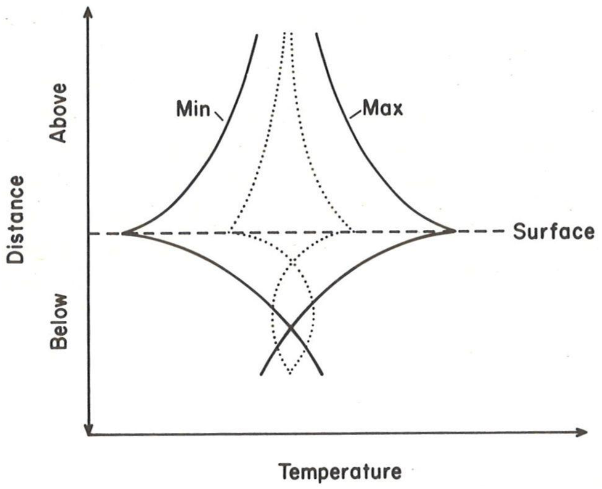


Figure 2.9: Temperature profiles above and below the active surface in times of max, min and intermediate (dotted line) surface temperature (Lee 1978:28)

As a logical consequence, thick leaf litter bodies should provide a broad variety of temperature conditions for ticks, depending on the radiation absorption at the surface and heat flux processes from the surface to deeper layers (see Chapter 4.5.3).

Not only the reduced radiation leads to cooler conditions in forest stands compared to the open field, but also, to a certain extent, a higher heat consumption through evapotranspiration processes (Otto 1994), depending on the actual water availability. The term *evapotranspiration* combines two kinds of evaporation processes. Via (1) *transpiration* the plant loses water from its interior, while the evaporation of precipitation from a plant or ground surface is called (2) *interception*. The expended energy of this phase changing processes (liquid → vapor) is called *latent heat* (Lee 1978).

Besides the warming through radiation exchanges on the ground surface, the temperature of the soil below is influenced by air exchange processes between soil and atmosphere, by the temperature of infiltrating water (for example rain) and by its ability to absorb the incoming energy, called thermal capacity (Geiger et al. 2009).

In winter, ticks overwinter in the litter and upper soil layer, their mortality rate closely connected to the conditions in this microhabitat. Due to the defoliation of trees in winter, the sheltering function of the canopy is highly reduced in broad-leaved and mixed forest stands. Consequently, a higher nocturnal radiation connected with the cooling of the forest floor can lead to litter frost and soil freezing, which are most prominent at clearings. These processes are restricted if snow covers the ground, because it shelters the forest floor from changes in air temperature, its insulating properties increasing along with its depth (Kreyling & Henry 2011). Then conditions revolve: Coniferous forests are now more exposed to soil frost than broad-leaved and mixed forests because its evergreen canopy prevents snow accumulation on the ground (Shanley & Chalmers 1999). However, intensive snow cover is restricted to higher regions in Baden-Württemberg, such as the *Schwarzwald* and *Schwäbische Alb*, and naturally less frequent in all other parts. In addition, soil frost is not only a matter of absent shelter but also of temperatures. In the last decades, minimum soil temperatures per year have increased and are expected to increase even more in the future and, accordingly, the days of soil freezing will decrease (Kreyling & Henry 2011). As a part of this thesis (Chapter 3.8.1 and 4.3.2), possible tick mortality factors are determined for the second winter of the period of data collection.

2.2.3 PROCESSES INFLUENCING MOISTURE

The water balance of a forest stand is usually a result of several processes influencing the amount of incoming and stored precipitation, according to the following equation (Otto 1994):

$$P - I = T + I + Ro + F + Rv$$

P = precipitation

I = interception: evaporation from plant or ground surface (left: from canopy leafs, right: from plant and ground surfaces beneath the canopy)

T = transpiration: evaporation from the plant itself

Ro = runoff of or near the ground surface

F = infiltration of water into the deep soil

Rv = reserve: storage of water (humus, shallow soil layers)

Water flow within the forest:

$$P = I + M + Pi + L$$

P = precipitation

I = interception: evaporation from plant and ground surfaces beneath the canopy

M = air moisture

Pi = directly incoming precipitation

L = leaf induced drop of water and flow along the tree trunk

Air moisture (= M) is mostly highest on the forest ground and lowest at the canopy, following a decreasing gradient (Lee 1978). Since the temperature shows a similar distribution pattern and the relative humidity is highly temperature dependent, (Geiger et al. 2009) conclude that relative humidity conditions within the stand (= M) are mainly a product of the temperature distribution and, to a lesser extent, are affected by incoming atmospheric air masses. The maximum on the ground can be explained by a higher water vapor providing due to evaporation (= I; passive, on the surface) and plant induced transpiration processes (= T) and by reduced mixing with dryer air masses. The ground therefore is more or less constantly moist (Geiger et al. 2009).

The soil moisture that emerges from precipitation has a stand specific, spatial variability due to precipitation distribution patterns through the canopy (= L) and, in addition, is reduced compared to the conditions in the open field. The soil corpus (= Rv) can partially retain incoming water, its extent depending on soil specific properties (Otto 1994). However, soil evaporation and temperature (average and amplitude) are reduced if the soil is protected by a covering layer, its extent growing along with the thickness of the layer (Bussière & Cellier 1994).

This context is of course also transferable to the upper organic layer, the humus. The humus is a protective layer above the ground, storage for water and heat (= Rv, see also (Ogée & Brunet 2002) and provides shelter for many organisms. It is a microhabitat of its own specific conditions, which will be presented in detail in the next chapter.

2.2.4 HUMUS AND LITTER

Moisture and temperature conditions within the humus and shallow soil layer affect *Ixodes ricinus* ticks in their inactive (hibernation, diapause, development) and resting phase, which together represent the major part of a tick’s life span. Surprisingly, little has been done to explore the tick's main habitat and thus the conditions ticks are actually exposed to most of their lifetime. The following chapter aims to give a short overview of relevant aspects.

The litter of a forest stand is mainly composed of leafs, dead (herbal) plant matter, twigs and fruit remains which are accumulated on the soil surface. The litter body acts as an insulating interface partly protecting the soil from direct atmospheric input such as rain, solar radiation and from temperature changes and, through the influence of all this input, creates a particular microclimate (reviewed in Sayer 2006).

The main litter mass in forests originates from defoliation processes in autumn. After winter, decomposition processes start when temperature reaches sufficient levels. The rate of decomposition generally increases with rising temperature and moisture up to an optimum limit but depends also on litter chemical composition and on characteristics of decomposing organisms (Moore 1986, Couteaux 1995, Berg 2014).

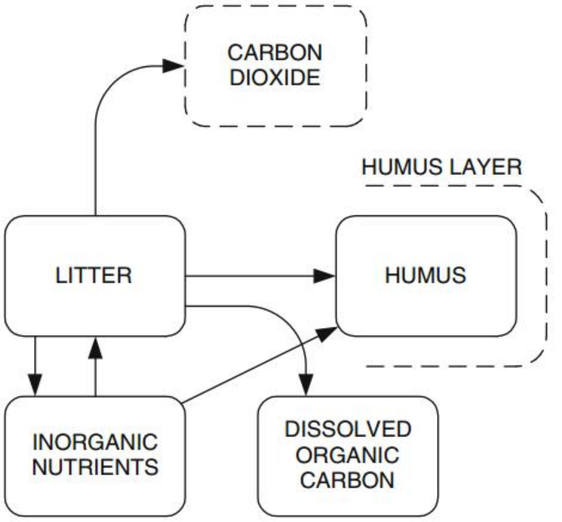


Figure 2.10: Generalized decomposition pathways. The microbiological decomposition transforms the leaf litter into humus and inorganic C (Berg 2014).

In general, litter of coniferous trees decompose slower than those of broad-leaved trees (Kang et al. 2009) but decomposition is of course also a matter of species. For example, leaves of the common beech (*Fagus sylvatica*) showed a lesser relative weight loss due to decomposition (0.4 +- 0.1%) compared to other deciduous trees like maple (*Acer pseudoplatanus*: 4.6 +-0.3%) or hornbeam (*Carpinus betulus*: 2.2+- 0.2) under similar climatic conditions (Cornelissen 1996). However, decomposition is a complex process which depends simultaneously on litter specific and environmental factors and these vary strongly over the years (reviewed in Berg & Laskowski 2005) which limits the significance of leave-species specific characteristics.

Mass losses in the litter depend also on the faunistic species composition. While earthworms tend to transport the litter from the soil surface into deeper mineral layers, the main litter decomposition is carried within the organic litter layer above the ground by arthropods like woodlouse, millipede and fly larvae as well as fungi (Sponagel 2005). Through these decomposition and transport processes, the litter incorporates into the soil and has a strong influence on its structure and stability as a major part of soil organic matter (reviewed in Sayer 2006).

The following part presents definitions and nomenclature used in soil science, partly adapted for the specific research objective of this thesis.

In the following, the term "**litter (layer)**" is used for the body of newly shed, not or partly decomposed organic substances above the mineral soil (L-/Of-layer). The "**humus (layer)**" describes the entity of organic substances on (L-l.) and within (Of and Oh-layer) the upper mineral soil. The underlying layer, which contains mainly mineral compounds and partly fine organic substances is the first mineral soil layer and is called **Ah** (Sponagel 2005, Berg & McClaugherty 2014).

The **L-layer** is build up by un-decomposed organic matter and is always located above the mineral ground surface. The **Of-layer** is built up by partly decomposed organic substances, which lay loose, packed or connected via fungi, fine roots or fine organic residuals on the ground. The proportion of fine substances normally increases with depth. The **Oh-layer** is built up by intense decomposed organic matter and often heavily infused by fine roots. It often contains mineral compounds to a higher extent (Sponagel 2005).

Depending on the layer composition, three main humus types are distinguished:

1) mull	L/Ah L/Of/Ah	Fast litter decomposition characterizes the mull type humus. Decomposition is mainly located in the mineral Ah-horizon (via earthworms and arthropods). The L-horizon compounds can be consumed before the next defoliation period, if conditions are suitable.
2) moder	L/Of/(Oh)/Ah	Litter decomposition is slow and located mainly in the humus layer (mainly via arthropods). Horizons are often highly intertwined.
3) raw humus	L/Of/Oh/Ah	The litter decomposition is restricted to the humus layer and remains often incomplete. Since litter transporting organisms (earthworms, arthropods) are missing the humus horizons are clearly separated from each other.

Each litter type as well as each litter layer has specific capacities to **store incoming precipitation** and to **minimize losses due to evaporation**. Sato et al. (2004) examined the moisture conditions of the L-layers of two forest types after rainfall (simulated) and found that the amount of retained rainwater was higher in broad-leaf litter than in needle-leaf litter and, of course, that the interception storage capacity increased along with the amount of precipitation. Furthermore, the interception storage capacity increases along with a growing mass of forest floor cover (litter or understory) and an increasing litter thickness, respectively (Putuhena & Cordery 1996, Park et al 1998). Park et al. (1998) found that not only the amount of stored liquid water but also the duration until all water is evaporated from the litter increases with increasing litter depth. At first, a water saturated litter layer (mixed forest) has a very small resistance to evaporation. With decreasing water content and an increasing amount of aerated pores within the litter layer, the resistance increases.

However, moisture and heat processes within the humus layer are very complex and difficult to evaluate due to the high variability of influencing parameters. To give an idea of the complexity: (Ogée & Brunet 2002) present over 90 (!) relevant substance characteristics and processes that are connected to their "forest floor model for heat and moisture including a litter layer". Moreover, the main part of litter layer related research is engaged with its decomposition and the release of nutrients and, compared to the latter, only little is done to examine the microclimate build by the litter layer itself (Scopus search of „leaf litter & microclimate“: 135 , „leaf litter & climate“: 871, „leaf litter & decomposition“: 3657 document results, access on 2016-03-01). Since *I. ricinus* ticks spend the majority of their lifetime sheltered near the ground, it seems of fundamental importance to gather new insights into microclimatic patterns provided by woodland litter layers. The combination of knowledge about the tick's physiology and the nature of their main microhabitat then allows evaluation, for example, of the often stated hypothesis that ticks are in general able to regain water losses and to describe related influence factors, processes and restrictions.

2.3 STUDY AREA

The study region is Baden-Württemberg with an area of 35,750 km^2. It is located in the South-West of Germany (Figure 2.11a). The altitude above sea level (a.s.l.) ranges from 85 m (Mannheim) to 1493 m (Feldberg) (Brachat-Schwarz & Manzke 2000). Figure 2.11b illustrates this altitude distribution. The natural vegetation of the state naturally would be woodland with European beech (Fagus sylvatica) as the main tree species (Otto 1994). But today the natural landscape is replaced by urbanized areas, agriculture and economically used forests (see Figure 2.11c). Forests cover 38 % of the state's area. Here, the main tree species is the economically important European spruce (*Picea abies*) with 38% followed by European beech with 21% of the total forest area (Statistisches Landesamt Baden-Württemberg 2012). Forests are mostly restricted to areas that offer unsuitable conditions for other usages. Thus, in areas with suitable conditions forests are often highly fragmented and surrounded by agricultural and built-up areas.

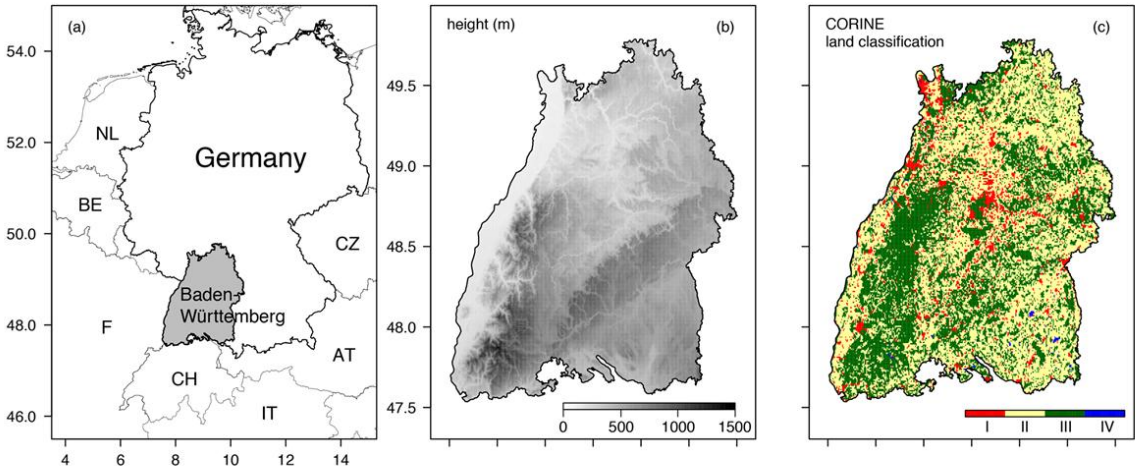


Figure 2.11: Baden-Württemberg: position, physical map and map of CORINE land classifications (I - Urban areas, II – Agriculture, III – Forests, IV – Water bodies) (Boehnke et al. 2015).

The climate can be classified using the Köppen-Geiger climate classification as Cfb (C = warm temperate climate, f = fully humid, b = warm summers) (Kottek et al. 2006). The annual average temperature varies between 3.5° and 11.5°C, the total annual precipitation between 600 and 2200 mm within the reference period of 1971 – 2000 (see Figure 2.12). Precipitation is frequent in all seasons (no dry periods according to the definition of Köppen-Geiger), but show a maxima in summer and winter and is lower in spring and autumn (DWD & LUBW 2006).

Baden-Württemberg is characterized by a high diversity of ecological conditions. The term "ecological landscape" is used for the german "Naturraum" which describes a unit of a geographical area with certain ecological conditions with respect to altitude, climate, natural vegetation, geology and geomorphology (Meynen & Schmithüsen 1962). Following the authors, the landscape of Baden-Württemberg is subdivided into 13 third-order ecological landscapes.

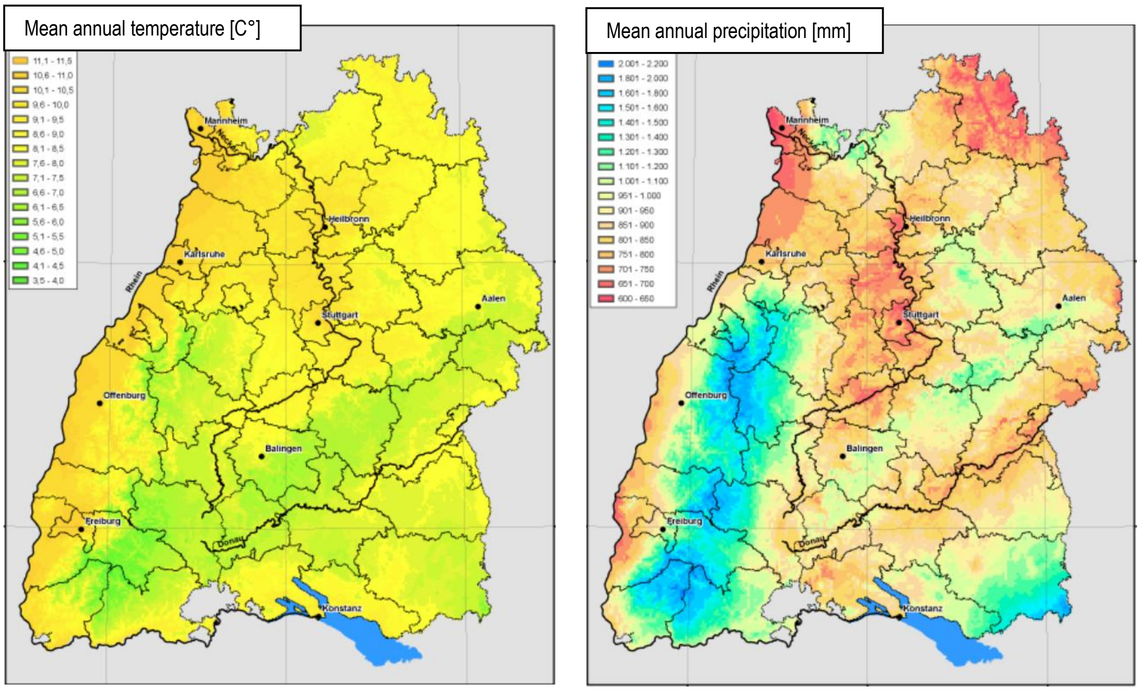


Figure 2.12: Maps of the 30 year mean annual temperature and precipitation distribution in Baden-Württemberg (source: http://www2.lubw.baden-wuerttemberg.de/public/abt5/klimaatlas_bw/ klima/ karten/index.html; accessed on 16th Sep 2015).

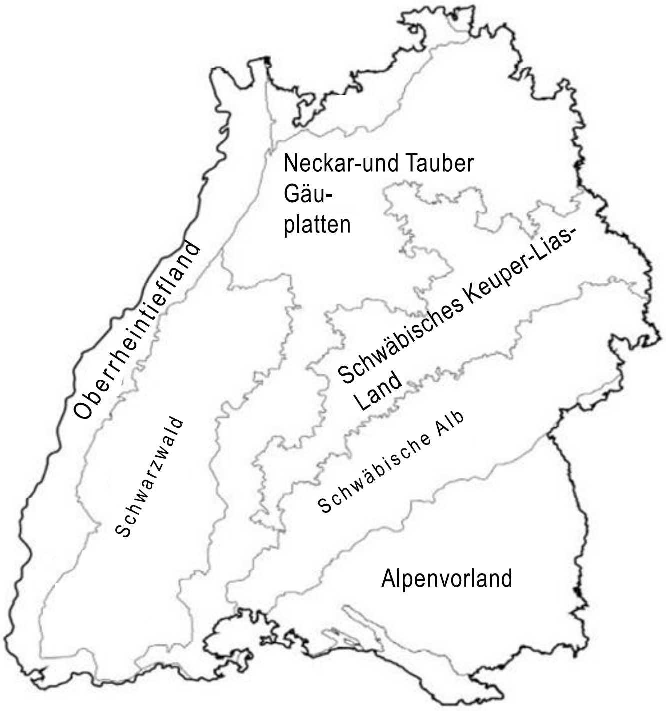


Figure 2.13: Ecological landscapes (Naturräume) in Baden-Württemberg used in this thesis (source of the shapes: http://udo.lubw.baden-wuerttemberg.de/public/).

To simplify this multiplicity in this thesis, the three third-order landscapes *Nördliches Oberrheintiefland* (northern), *Mittleres Oberrheintiefland* (medium) and *Südliches Oberrheintiefland* (southern) are combined into one ecological landscape: the *Oberrheintiefland*. Furthermore, the two third-order landscapes *Donau-Iller-Lech-Platte* and *Voralpines Hügel-und Moorland* are combined into the *Alpenvorland*, which is the generic term for the second order landscape of this region (LUBW 2010a). Figure 2.13 illustrates the partitioning of ecological landscapes used in this thesis.

As one of the warmest regions in Germany, the *Oberrheintiefland* forms the Western border of Baden-Württemberg. On average, a mean annual temperature of nearly 11°C and a low annual precipitation of 600 mm are typical. The altitude ranges from 85 m a.s.l. in the North to 245 m a.s.l. in the South. To the East, the low mountain range *Schwarzwald* adjoins, with the highest mountain *Feldberg* (1493 m a.s.l., 3.3°C, 2000 mm). The second lowest mountain range is the *Schwäbische Alb*, a plateau that declines to the East with the highest point near *Gosheim* (up to 1015 m a.s.l.). The rest of the country is characterized by mostly hilly areas of intermediate altitudes and a regional climate which is influenced by the bordering mountains, e.g., in matters of precipitation level and distribution (*Neckar- und Tauber Gäuplatten, Schwäbisches Keuper-Lias-Land, Voralpines Hügel- und Moorland, Donau-Iller-Lech-Platte*) (Siegfried Kullen 1984, LUBW 2001, DWD & LUBW 2006). In these landscapes, forests are restricted to areas unsuitable for agriculture or housing and thus highly fragmented. In contrast, the largest connected wooded areas are located in the low mountain range *Schwarzwald* where poor soil quality and steep slopes prevent other usages. Coniferous and mixed stands are dominant (Figure 2.11c) (LUBW 2001). On a long-term average (1971-2000), monthly precipitation amounts are in general highest in summer and also raised in winter, especially in landscapes at medium to high altitude, e.g. *Schwarzwald* (DWD & LUBW 2006).

Ixodes ricinus ticks are distributed all over Germany (see Figure 2.1) but, in contrast, the tick-borne encephalitis virus seems to concentrate in the Southern states Baden-Württemberg and Bavaria and to be widely absent in Northern states (see Figure 2.14). Over the last decade, all administrative districts of Baden-Württemberg were defined as tick-borne encephalitis (abbr.: TBE) risk areas by the definition of the Robert-Koch Institute, Berlin: “A district is defined as a risk area if more than one TBE case per 100.000 residents were reported over a five-year period” (Robert Koch Institut 2015). The basis for those maps is the German Law on the Prevention and Control of Infectious Diseases (Infektionsschutzgesetz), which states that a laboratory detected TBE virus must be reported to the health authorities since 2001. In contrast to TBE, there is no German law that promotes the report of human Lyme disease cases, so that the risk of a Borrelia infection has to be estimated by tick infection rates. Kimmig & Oehme (2009) expect Borrelia bacteria to be present all over Germany and, according to a tick infection rate of 15 % on average (TBE = 1-2 %) and local maxima of about 40 %, Lyme disease cases to be much more frequent than TBE. Interestingly, the number of TBE cases in an risk area is not uniformely distributed over Baden-Württemberg. Most cases were reported in districts in the *Schwarzwald* region and bordering landscapes, near *lake constance* and the city of *Heidelberg* (Schick 2009).

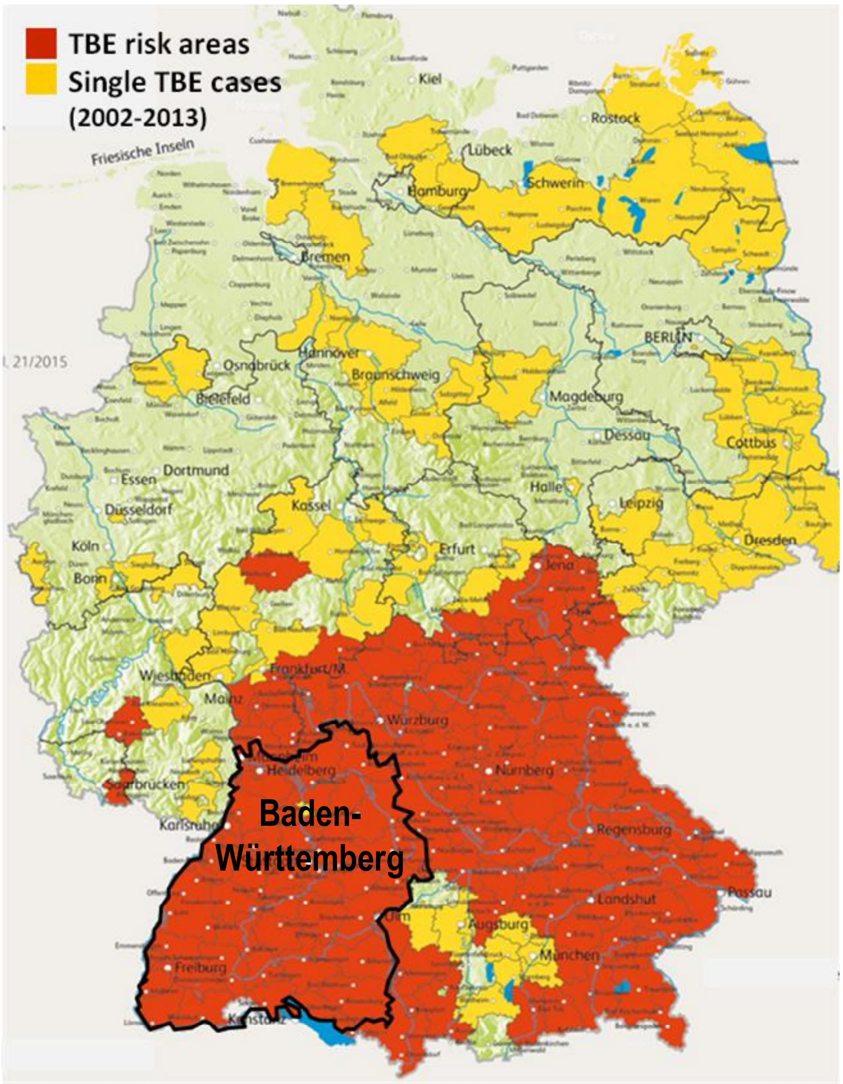


Figure 2.14: TBE-risk areas in Germany by the definition of the Robert-Koch Institute (Robert Koch Institut 2015) (Source of graphic: modified after (Pfizer Pharma GmbH 2015)

3 Materials and methods

3.1 STUDY SITES

The selection of the 25 study sites focused on the large-scale distribution of ticks and their pathogen prevalence. For this reason, most study sites were selected due to the expectation of a high suitability for ticks.

In a first step, areas were identified which

- are typical for their surroundings
- represent altogether the ecological variability of Baden-Württemberg

by use of maps and sources listed in Table 3.1.

The **ecological conditions** for all 25 study sites were chosen as varied as possible regarding to altitude, mean annual temperature, moisture and soil type, in order to represent the entire regional variability range of the study area Baden-Württemberg.
In a second step, the **habitat type** was selected. Current knowledge indicates that mainly forests provide sufficient conditions for *Ixodes ricinus* populations in Central Europe, although they also occur in other habitats. Therefore, the examination focuses on forest and forest-like habitats.
Therefore, a set of different forest types where chosen including broad-leaved, coniferous and mixed forests.
On the one hand, the forest type of a study site should be representative for its ecological landscape. Therefore, the publicly available information given by the *Landesanstalt für Umwelt, Messungen und Naturschutz Baden-Württemberg* about characteristics of each ecological landscape was considered (LUBW 2001). Figure 3.1 depicts the area distribution of land use, forest type and forest fragmentation for the six largest ecological landscapes (illustrated in Figure 2.13). On the other hand, habitats characterized by specific conditions were also included. For example, to test the hypothesis that habitats with moist soil conditions (located in a depression area or near water bodies) are inhabited by comparably high numbers of ticks. The

hypothesis is based on knowledge gathered at a preliminary study (Boehnke 2011). Only corporate and state-owned forests were taken into account.

Table 3.1: List of maps and sources used for the selection of the study sites.

subject	source name	reference
natural landscapes	*Naturräume Baden-Württembergs*	Meynen & Schmithüsen 1962, LUBW 2010b
	Naturraum	Daten- und Kartendienst, LUBW
altitude	*Google Earth, altitude (m)*	Google Earth
topography/relief	*Topographische Karte / Relief (DGM 5)*	Daten- und Kartendienst LUBW
climate	*Übersichtskarten*	(LUBW, 2006)
	Klimakarten Baden-Württembergs (p.23)	Brachat-Schwarz & Manzke 2000
soil	*Bodenübersichtskarte der BRD (BUEK*	BGR 1995
geology	*Geologische Karte Baden-Württembergs*	Brachat-Schwarz & Manzke 2000
groundwater	*Hydrogeologische Einheit*	Daten- und Kartendienst LUBW
land use	*Landnutzung (Landsat 2000)*	Daten- und Kartendienst LUBW
natural vegetation	*Potentielle natürliche Vegetation*	Daten- und Kartendienst LUBW

During the field examinations, forest areas with a **homogeneous stand character** and a **high suitability for the tick sampling method** were chosen. For example, forests were avoided if they contain thick bush and young tree cover or a prickle-rich understory (e.g. *Rubus spp.*) that hamper tick sampling.

The chosen forest habitats usually have a distinct stand and crown density leading to particular climatic conditions within the stand compared to the surrounding atmosphere. For additional variation, a **forest with a thin stand density** (**ST**) and a **wide clearing** (**RF**) were chosen as well as a thin stand in a protected **swamp** (**WU**).

One **ravine** (**HQ**) highly covered by young beech trees provides particularly cool and moist conditions. Three **floodplain forests** (**AL, AW, NA**) are included as well as three **forests in a depression area** (**FN, ST, VS**) with temporarily very moist ground conditions.

One aspect that can influence tick densities especially in hilly and mountainous regions is the exposure to the sun ,e.g., south- or sorth-facing slopes (Lane et al. 1985). To reduce the impact of local scaled effects (definition in Chapter 2.2.1), most sites are situated on an even terrain with no distinct sun exposure. To include those effects, some sites are situated on a **southern** (**FR**) or **northern slope** (**CW, WR**), representing common situations of their ecological region.

A detailed study site description including microclimatic time series is given in Appendix (A).

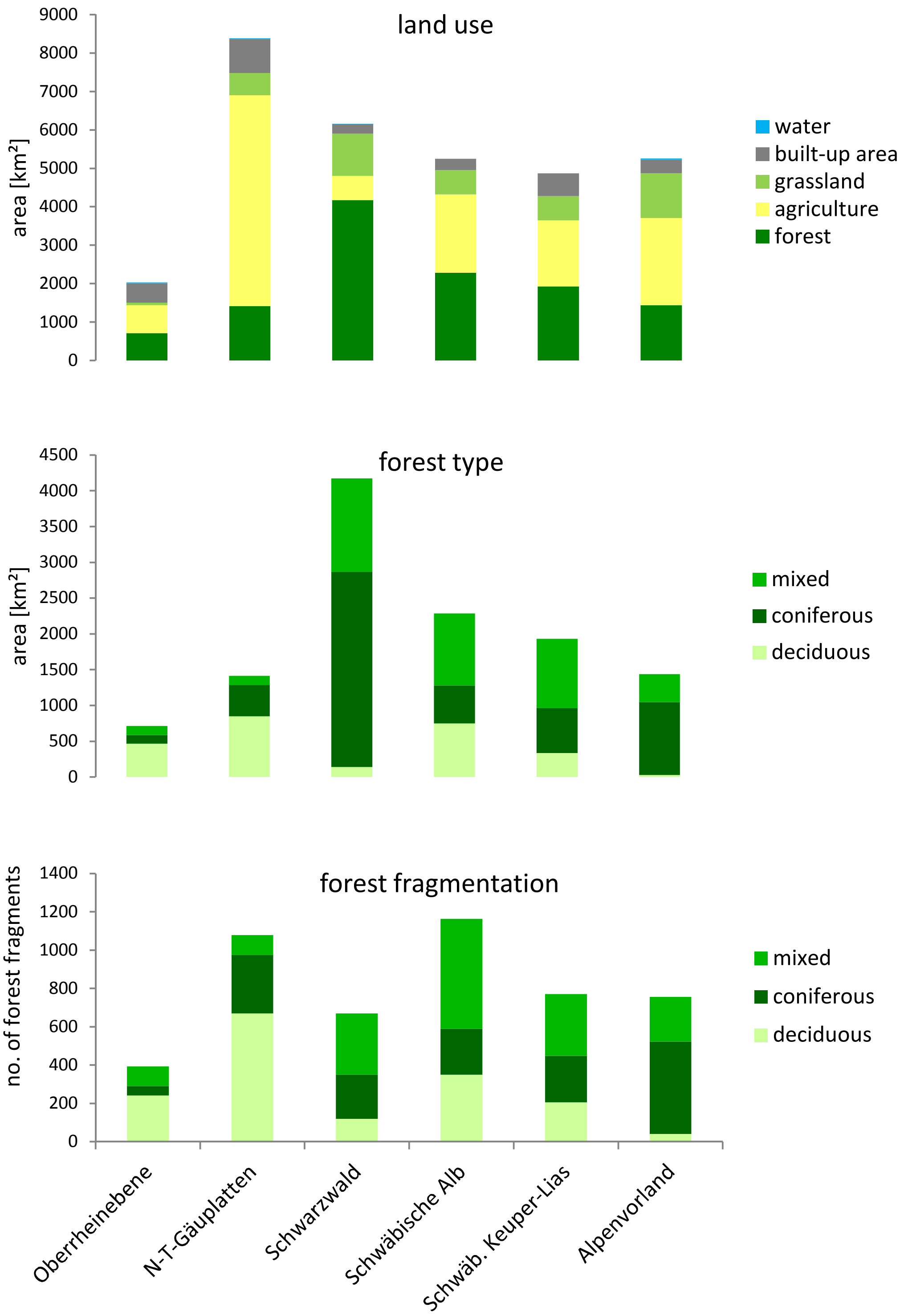


Figure 3.1: Area distribution of land use, forest type and forest fragmentation for the ecological landscapes in BW. The *Schwarzwald* has the widest forest areas, while in contrast a high number of isolated forest patches are to be found in *Neckar-Tauber-Gäuplatten* and *Schwäbische Alb*. Data: CORINE 2010.

Field examinations were partly carried out in collaboration with Stefan Norra, Reiner Gebhardt, Trevor Petney, Miriam Pfäffle and Nina Littwin (Western Baden-Württemberg), as well as Patrick Sebastian and Rainer Oehme (Eastern Baden-Württemberg). Figure 3.2 depicts the position of the 25 study sites over the study region Baden-Württemberg. Additionally, forest type and kind of microclimatic measurement setting is labeled. Figure 3.3 illustrates the number of study sites according to their forest type and the associated ecological landscape.

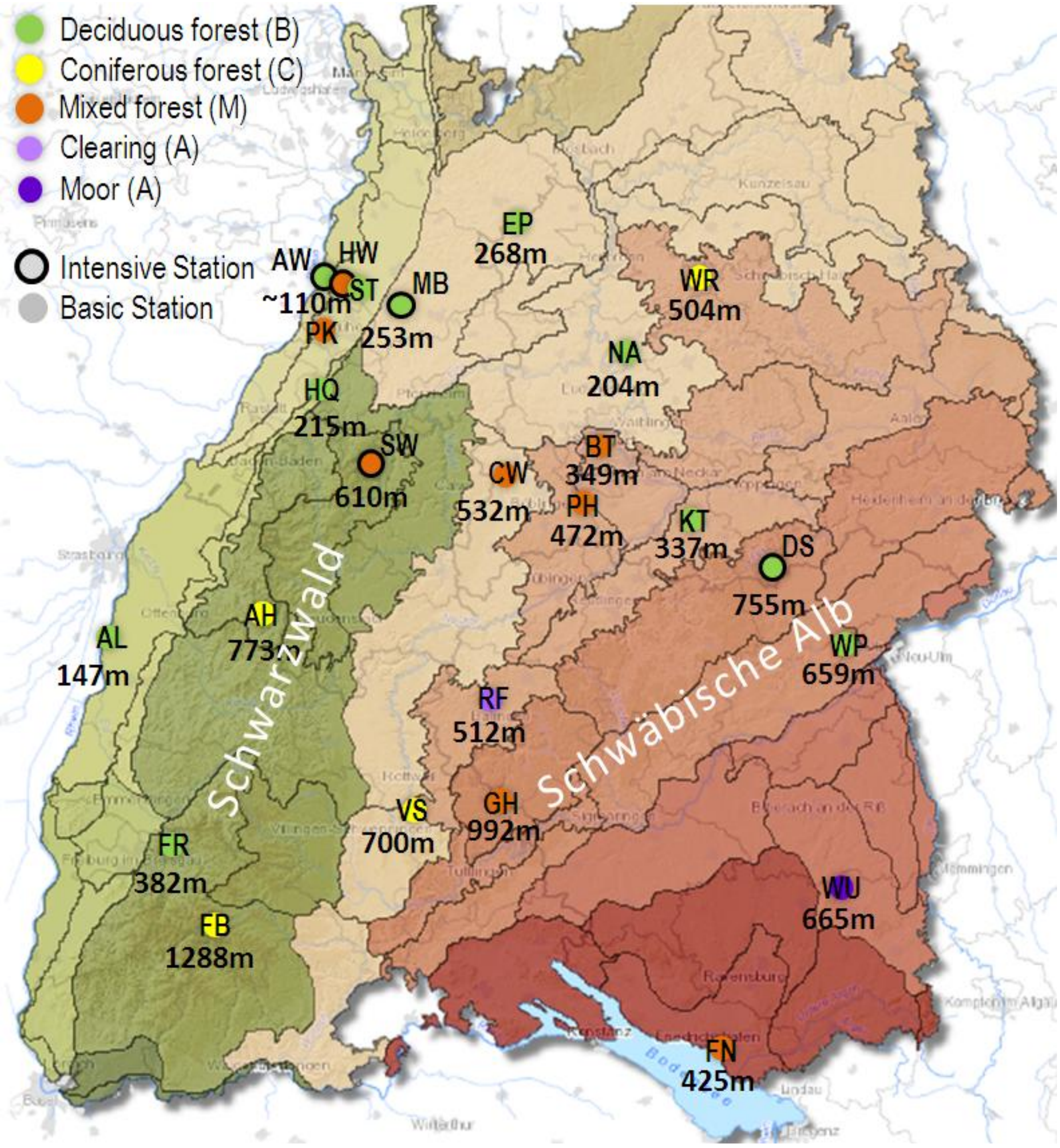


Figure 3.2: Positions, altitude in m a.s.l., forest type and station setting of all 25 study sites within the area of Baden-Württemberg. The background map highlights different ecological landscapes, two of them are labeled for illustration purposes (source: map from LUBW, Naturräumliche Gliederung, modified).

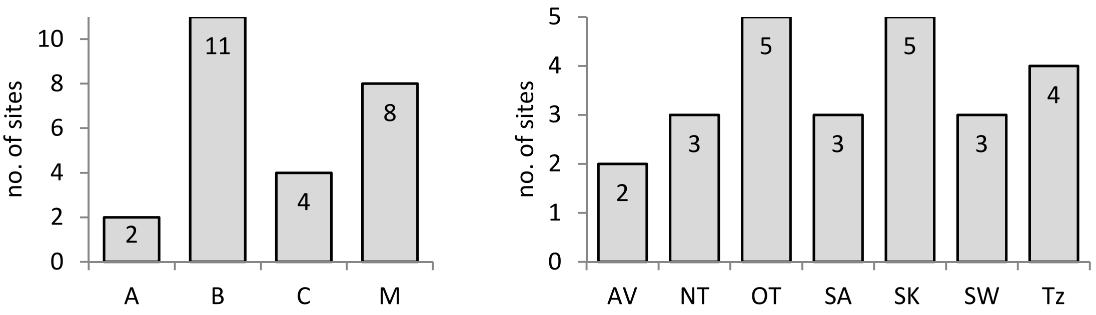


Figure 3.3: Number of study sites according to forest type (left; C = coniferous forest, B = deciduous/broad-leaved forest, M = mixed forest, A = others) and ecological landscape (right; AV = Alpenvorland, NT = Neckar-Tauber-Gäuplatten, OT = Oberrheintiefland, SA = Schwäbische Alb, SK = Schwäbisches Keuper-Lias-Land, SW = Schwarzwald; Tz = site is located in a transition zone (HQ, FR,VS,MB)).

3.2 MICROCLIMATE

The environmental factors temperature and moisture, especially relative humidity and saturation deficit, have a major influence on tick behavior, development and survival success (see Chapter 2.1.4). The main habitat of ticks are forests, where they live mostly near the ground surface and where climatic conditions differ considerably from conditions measured by official weather stations (see Chapter 2.2). Since one aim of this study is to analyse the impact of environmental factors on ticks, it is necessary to collect our own climate data in the microhabitat of this species, following the requirements stated in (Estrada-Peña et al. 2013). In the year 2010, a first explorative examination of the influence of microclimate and habitat characteristics on tick abundances and activity was carried out at five study sites North of Karlsruhe. Temperature was measured in the soil, at 25 cm and 50 cm above the ground, relative humidity at 50 cm. There was hardly any difference between the temperature at 25 cm and 50 cm within a single site, and no significant differences between the five sites. However, there was strong evidence that water availability and groundwater depth pose a strong influence on *I. ricinus* abundances (Boehnke 2011).

Based on this knowledge and with regard to project demands, two different microclimatic measurement settings were developed in collaboration with Stefan Norra, Trevor Petney and Reiner Gebhardt. One setting is reduced to the main microclimatic parameters for large-scale examinations (Chapter 3.2.1.: Basic measurement), the other one is expanded to depict the microclimatic variability within a forest stand (Chapter 3.2.2.: Intensive measurement). At the Intensive Stations AW, HW, MB, SW and DS, the parameters air temperature and relative humidity are measured at two spots, soil temperature at 4 spots. To make these data comparable to the singularly Basic Station results, similar parameters were averaged on a 10 min resolution basis.

Table 3.2: Overview of measured parameters at each study site. Measurement ended in autumn 2015.

	measurement starting time		50 cm	50 cm	5 cm depth	5 cm depth	litter zone	litter zone	2 m, inside	2 m, inside	2 m, outside	2 m, outside	2 m, outside	2 m, outside	2 m, outside	2 m, outside
Abbr.	**Basic**	**Intensive**	**T_A**	**RH_A**	**T_S**	**SM**	**T_L**	**RH_L**	**T_I**	**RH_I**	**T_O**	**RH_O**	**AP**	**wind**	**prec**	**GR**
AH	01.08.12		■	■	■	■										
AL	01.08.12		■	■	■	■										
AW	18.07.12	24.10.12	■	■	■	■	■	■	■	■	■	■	■	■	■	■
BT	09.05.13		■	■	■	■										
CW	12.09.12		■	■	■	■										
DS	20.07.12	18.12.12	■	■	■	■	■	■	■	■	■	■	■	■	■	■
EP	27.07.12		■	■	■	■										
FB	03.08.12		■	■	■	■										
FN	07.09.12		■	■	■	■										
FR	12.05.13		■	■	■	■										
GH	06.09.12		■	■	■	■	░	░								
HQ	19.04.13		■	■	■	■										
HW	17.07.12	25.10.12	■	■	■	■	■	■	■	■	■	■	■	■	■	■
KT	28.07.12		■	■	■	■										
MB	18.07.12	25.10.12	■	■	■	■	■	■	■	■	■	■	■	■	■	■
NA	27.07.12		■	■	■	■										
PH	10.07.13		■	■	■	■										
PK	01.05.13		■	■	■	■										
RF	06.09.12		■	■	■	■										
ST	01.08.12		■	■	■	■	░	░								
SW	25.07.12	16.11.12	■	■	■	■	■	■	■	■	■	■	■	■	■	■
VS	06.09.12		■	■	■	■										
WP	28.07.12		■	■	■	■										
WR	27.07.12		■	■	■	■										
WU	07.09.12		■	■	■	■										

T	temperature	A	air	Starting time of litter measures:		
RH	relative humidity	S	soil	DS: 26.2.2013		
SM	soil moisture	L	litter	SW: 21.3.2013		
AP	air pressure	I	inside the forest	AW: 11.4.2013	■	regular measure
prec	precipitation	O	outside the forest	HW: 11.4.2013	░	complementary measure
GR	global radiation			MB: 11.4.2013	□	no measure

Table 3.2 gives an overview of measured parameters at each study site. A technical description of the equipment is given in Appendix (B1).

The equipment selection, the assembly of single components, the setting of the software solutions for read-out and data conversion, as well as sensor calibration was mainly executed by Reiner Gebhardt under the assistance of the author.

3.2.1 BASIC MEASUREMENT – GEOGRAPHICAL RANGE

At twenty so called “Basic Sites”, data loggers measured and stored data of four different microclimatic parameters (Table 3.2). Air-temperature (T_A) and relative humidity (RH) sensors protected by a white radiation shield measured at 50 cm above ground level (see Figure 3.4) to record the conditions actively questing ticks are exposed to. There are only a few studies in tick research that used a similar methodological approach before (for example Estrada-Peña et al. 2004, Boehnke 2011).

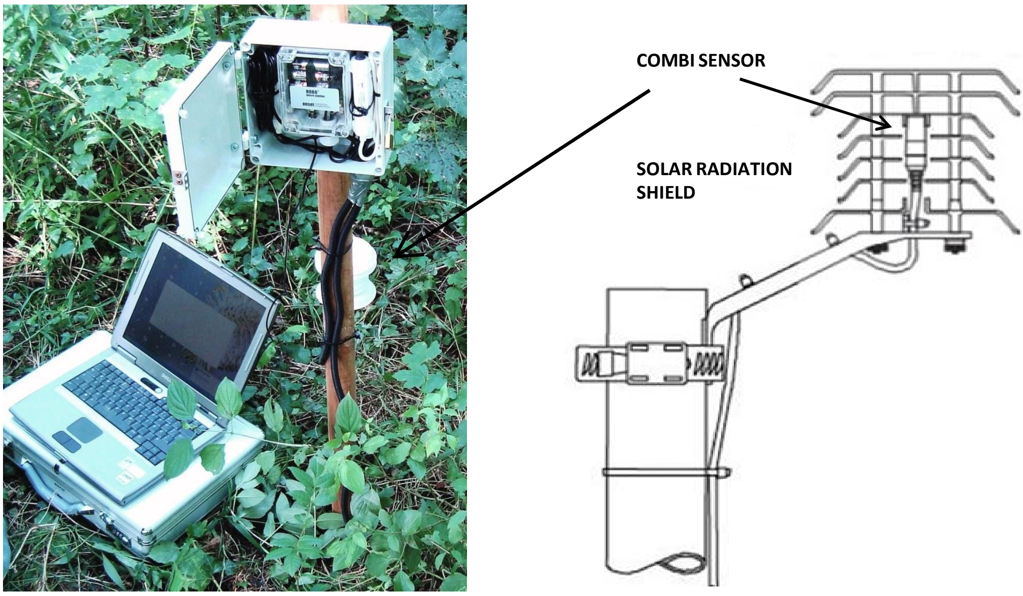


Figure 3.4: Picture of a **Basic Station** and the readout process in the field (left) and drawing of the solar radiation shield with the air temperature / relative humidity combi sensor (right, from www.onsetcomp.de, access on 5th Nov 2015). Soil temperature and moisture sensors are buried in 5 cm soil depth.

Data were stored as 10 minute average values. We calibrated all sensors before usage, and donated the whole setup as a “Basic Station”.

3.2.2 INTENSIVE MEASUREMENT – HABITAT RANGE

At five study sites an advanced measurement concept was used. The “Intensive Sites” are Auwald (AW), Hardtwald (HW), Michaelsberg (MB), Gaistal im Schwarzwald (SW) and Drackenstein (DS) (Table 3.2). Figure 3.5 illustrates the setting of the station and sensors in the field. gives an overview of the measured parameters. The sensors outside the forest were placed as far away as possible from the forest border to reduce horizontal limitation effects.

However, due to the large height of the trees and the limited space at the open field, it was not possible to place the sensors without any radiation reducing influence of the forest. The measurement concept was developed to examine the extent to which the measurements at 50 cm height and within 5 cm soil depth are representative. Therefore, at first, air temperature and relative humidity (via a combi sensor) were measured at three spots at 50 cm above the ground and soil temperature at four spots. However, first data explorations at the end of 2012 showed that there was hardly any difference between the conditions of the three spots at 50 cm height, at all five study sites. These findings offered the opportunity to rearrange one of the 50 cm height sensors to the litter zone, to gather new insights on the micro habitat where ticks develop and regain water losses (see Chapter 2.1.4).

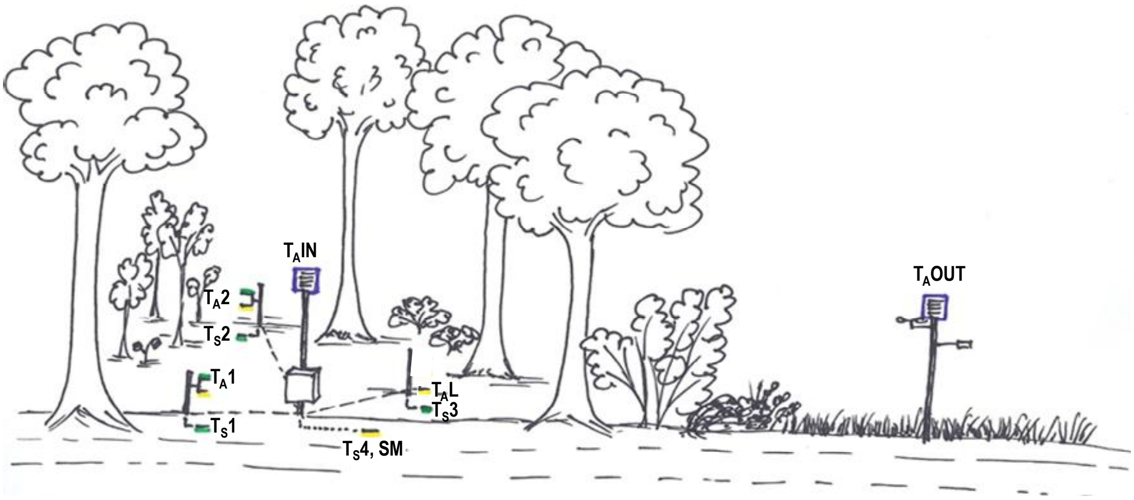


Figure 3.5: Drawing of the microclimatic measurement setting of the **Intensive Station** and exemplary labelling of sensors. All T_A-sensors are combined with RH-sensors ($T_A1 + RH_A1$).

At the Intensive Stations AW, HW, MB, SW and DS, the parameters air temperature and relative humidity are measured at two spots, soil temperature at 4 spots. To make these data comparable to the singularly Basic Station results, similar parameters were averaged on a 10 min resolution basis.

3.2.3 SPECIAL MEASUREMENT – GOSHEIM

Some sites showed notably high tick densities in 2013, which denote especially favorable habitat or host conditions there. One site showed a great promise for new insights into the importance of specific environmental conditions for tick survival. Thus, supplementary microclimatic measurements started in the following year.

Gosheim (GH) is the second highest study site after Feldberg (FB), and the highest point within the *Schwäbische Alb* (992 m a.s.l.). The *Basic Station* is located at the border of a small clearing within a medium-dense and coniferous dominated mixed forest stand. The total study area contains various sections with different tree densities, species compositions and clearing width due to forest management practices.

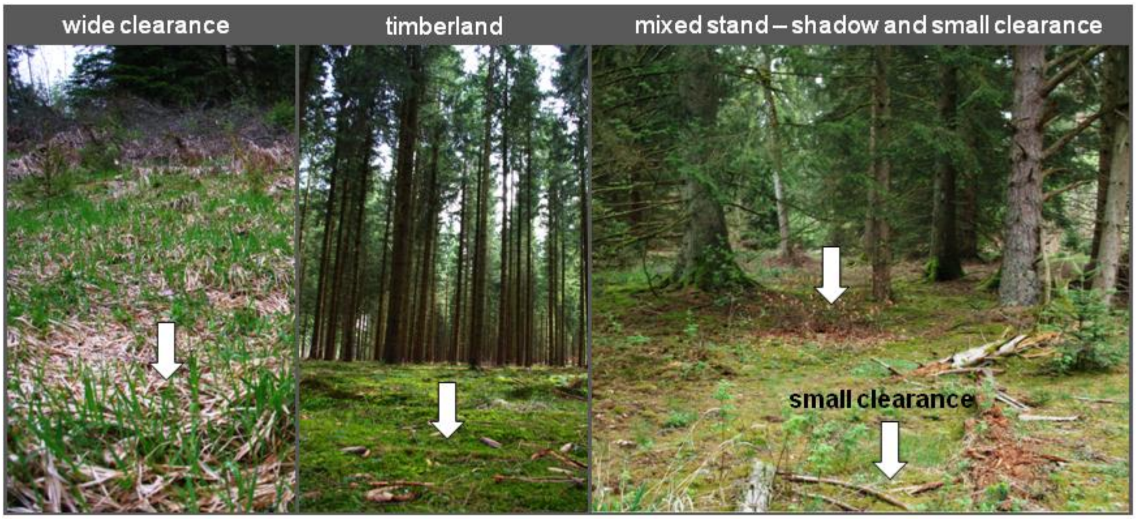


Figure 3.6: Pictures of the three different forest parts in Gosheim (GH) where loggers (location: white arrows) recorded additional temperature data within the range of the litter layer in 2015.

One problem for ticks near their altitude dependent distribution boundary is the **limited heat budget** throughout the season, which restricts successful development of all tick stages, and active host finding. Because of this, it was all the more surprising that all tick stages (larvae, nymphs and adults) were captured in 2013 with a high number of nymphs (32) compared to other sites located at high altitudes (for example AH with 773 m a.s.l. and 3 nymphs in 2013).

The detection of larvae is a strong evidence for a successful development of eggs within this habitat. **Tick development processes are strongly temperature dependent** and are, at the current state of knowledge, located within the litter layer or near the ground, respectively. Thus, additional measures (hourly means) were taken within the first centimeters above the ground surface using Escort Mini 2000 Temperature Logger, to examine the **temperature conditions in the litter layer** which ticks are exposed to at different parts of the forest from 2014-04-01 to 2014-08-31 (Figure 3.6). Figure 3.7 illustrates the installation of a temperature logger in the field, under moss.

Figure 3.7: Picture of a temperature logger placed under moss.

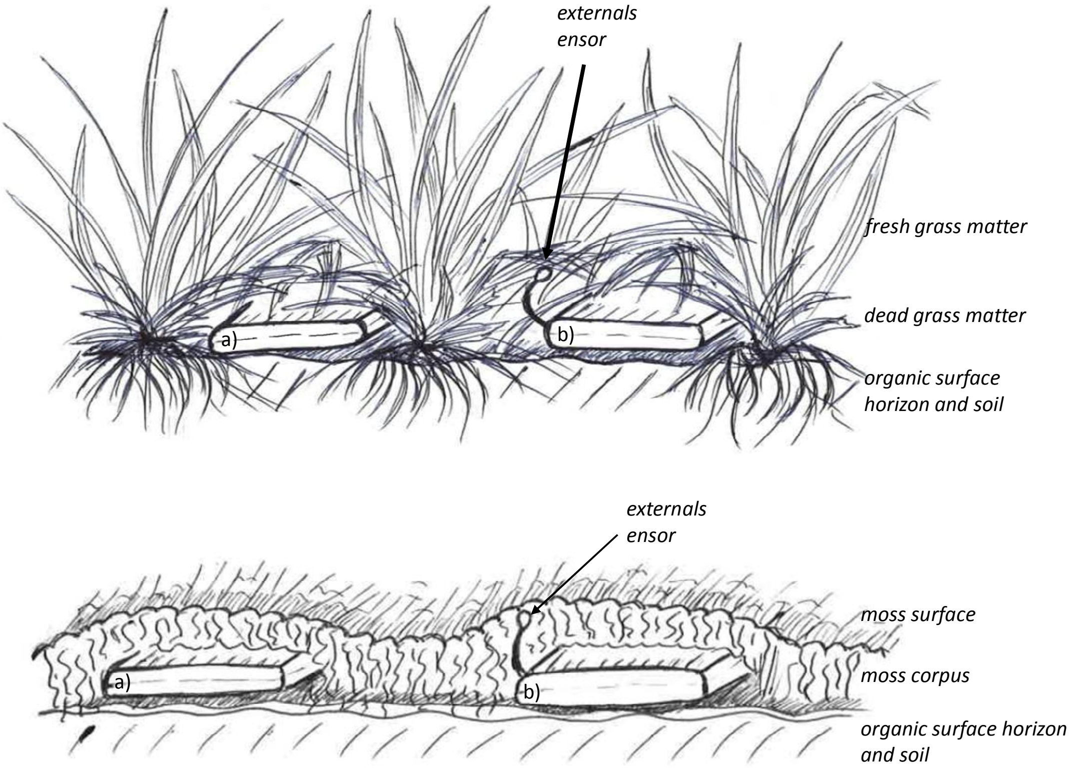


Figure 3.8: The graphics illustrate the installation of the Escort Temperature Logger in the grass matter (top) and the moss corpus (bottom) in the original (a) and modificated (b) configuration (author`s own drawing).

In the original configuration, the temperature sensor is located within the case and embedded in a heat-conducting paste. Thus, loggers detect temperature changes only after the whole case changed its temperature. To avoid such delayed recordings, I relocated the temperature sensor to the outside and thus modified the recording configuration of some loggers. Figure 3.8 illustrates both logger types, installed under moss or grass vegetation. Some modified loggers were operated together with original loggers to monitor the impact of configuration changes in the field. Temperatures within the grass matter were only measured at the wide clearing. All other measurements (in the timberland and mixed stand) were operated within the moss layer.

3.2.4 OFFICIAL WEATHER DATA

The German Weather Service (Deutscher Wetterdienst, DWD) operates a climatic measurement network with 182 full-time weather stations (status: Aug 2015) in Germany and provides several freely accessible climatic datasets online (http://www.dwd.de/DE/leistungen/cdcftp/cdcftp.html, last access on 14th Oct 2015). These stations are located in the open field, since data of official weather stations represent conditions of larger surroundings with preferably no environmental influences (Geiger et al. 2009).

In this thesis, air temperature (°C) and relative humidity (%) data were used, measured at 2 m height at ground stations. Comparisons between official weather data and microclimatic data from the study sites were made with hourly data of the nearest official weather stations. Table 3.3 provides a description of the official weather stations used in this thesis.

Table 3.3: List of official weather stations and associated study sites.

station name	station_ID	parameter	resol.	lon	lat	m a.s.l.	assoc. study sites
Rheinstetten	4177	Ta, RH	hourly	8.33	48.97	116	AW, HW, MB
Klippeneck	2638	Ta, RH	hourly	8.75	48.11	973	GH

Additionally, regional averages of Baden-Württemberg were used to calculate a year-on-year comparison of monthly average temperatures for the years 2013 and 2014.

Most other research on the influence of climate on ticks in their specific habitat was done exclusively on the basis of official weather data (for example: Perret et al. 2000, Hubálek et al. 2003, Perret et al. 2004, Li et al. 2012).

3.2.5 DATA PROCESSING

3.2.5.1 DATA STORAGE

Loggers at **Intensive Sites** recorded the data with a special coding that had to be converted in the office afterwards. Reiner Gebhardt executed all associated procedures and converted the data into Excel format for further processing. Accordingly, data of the **Basic Sites** were read out as .txt files and converted into Excel format. To obtain comparable data sets with a basic measurement structure, the doubled (Ta and RH in 50cm) or tripled (Ts) values of the *Intensive Sites* were reduced to one value by calculating 10 min means. Data preparation was done using Microsoft Excel (2007).

3.2.5.2 QUALITY ASSURANCE

The **Basic Stations** operated reliably looking at erroneous data caused by software problems. One major issue of all stations was the energy supply, which was provided by batteries stored inside the logger box. Extensive arrangements were undertaken to keep batteries, logger and serial interface between logger and sensors dry and fully functional; batteries and logger were stored in a box, which was stored together with the interface, cables and drying agents in a larger, waterproof box. Only cables stored within protective cable sheathing went outside the box. Sensitive transition areas between box and cable sheathing, and cable sheathing and sensors were additionally protected against bite damages. Despite these precautions, different loggers showed a high variability (3 month to > 1 year) in their lifespan of energy supply, provided by one set of batteries. Moreover, soil moisture measures (depending on direct soil contact) were disturbed when burrowing animals unearthed the sensors. Since data had to be read out in the field and checked for errors afterwards, many data gaps occurred at the beginning of operation in 2012 and 2013 due to occurrences of these specific problems. After increasing the frequency of the preventive maintenance visits, data losses were strongly reduced in 2014.

Looking at **Intensive Stations,** the complex software caused some issues. For example, single erroneous data points (outliers on a 10 min basis) had to be identified from time series and replaced individually by mean values of the surrounding, more reliable values. These operations were done using Microsoft Excel 2007 or R (R Core Team, Version R i386 3.0.1) via the user interface of RStudio. Additional problems were caused by soil temperature sensors, which lead to unrealisticly increasing temperatures in 2013. Hence, Reiner Gebhardt manufactured new sensors and replaced the defective sensors in the field.

The validity of the climatic data of **all stations** was checked on a time series basis, by comparisons with data from nearby stations and, if necessary, by information about weather situations from weather services. If, for example, the monthly-average temperature course in a day seemed unrealistic, data from nearby official weather stations or other survey stations were used as well as specific climatic descriptions from the German Weather Service (Deutscher Wetterdienst) to check the findings. However, since data were partially measured in well-shaded forests, at slopes with a specific sun exposure, in a ravine and so on, the degree of comparability to nearby stations and especially to official weather data is quite uncertain. Thus, to approximate the reliability of the data, sometimes complex considerations involving all available information had to be made. A good summary of the idea of quality control and the issues researchers are confronted with are given by Peterson et al. (1998) and Daly (2006). To reduce misinterpretations, tick related analyses were mainly based on data with a high degree of reliability. We checked data from Intensive Stations for outliers constantly on a visual basis, and tested data from Basis Stations if used for analysis and at random. To replace missing data on a daily average basis a concept was invented for the parameters air and soil temperature and relative humidity (see Chapter 3.6.1).

3.2.5.3 SATURATION DEFICIT

Ixodes ricinus ticks are very sensitive to dehydration. Relative humidity is the factor that describes the opportunity for ticks to uptake water actively from the unsaturated air. In contrast, water losses caused by a low air humidity are best described with the saturation deficit parameter, which influences tick behaviour and mortality due to its drying power (Perret 2003, Burri et al. 2007). In fact, the factor relative humidity as well as the saturation deficit both are influenced by temperature and describe two sides of the same coin. The saturation deficit SD is calculated from temperature (temp) and relative humidity (RH) data, following the Magnus formula (over liquid water) (Zmarsly et al. 1999):

a) $\mathrm{E = 6.1*10\hat{}((7.5*temp)/(temp+237.2))}$

b) $\mathrm{e = E*RH/100}$

c) $\mathrm{SD = E - e}$

with the saturation vapour pressure E (in hPa) and the dew-point temperature e (in °C).

3.2.5.4 VEGETATION PERIOD

One way to asses distribution boundaries of *Ixodes ricinus* ticks was described by Jaenson et al. (2009, 2011). They found that the Northern limit of this species can be characterized by an average vegetation period of about 170 days per year and an start of spring before May. It mainly occurs in regions with a vegetation period of 180 days and more. Baden-Württemberg is far away from this northern boundary, but has mountains that could outheight the altitude dependent distribution boundary of the tick. In this thesis, this approach is used to test if results are transferable to this task. The vegetation period is defined as the number of days between the end of the first continuous 4-day period with a 24-h mean temperature >5°C and the beginning of the last continuous 4-day period with a 24-h mean temperature >5°C (Jaenson et al. 2009).

3.3 VEGETATION, HUMUS AND HOSTS

Until now, there is no standard procedure to characterize forest habitats in consideration for the needs of *I. ricinus* ticks. Even worse, there seems to be no consensus on the influence of singular environmental factors. Thus, a **survey concept** had to be created first. According to (Tremp 2005), each vegetational survey has to be chosen with regard to its specific research question – which would be the relationship between tick abundance and vegetation. The following section contains single steps of the creation process.

Studying several publications lead to the impression that not the plant species itself, but rather the **physical conditions provided by plants** are responsible for tick presence or absence. On this issue important work was carried out by (Milne 1944).

The vegetation and its remnants have a great impact on the climate, especially the microclimate (physical conditions within forests as well as processes and underlying pattern are described in Chapter 2.2). Since development, behaviour and mortality of ticks are highly influenced by external, microclimatic conditions and, in addition, these microclimatic conditions are always bound to **vegetational structures**, the examination of these structures and their possible influence on ticks was a main aspect of this work.

Thus, a variety of potentially meaningful vegetational parameters for *I. ricinus* ticks were chosen on the basis of literature results (Chapter 2.1), field experience of the author as well as considerations regarding microclimatic pattern (Chapter 2.2). In addition, literature relating to the issues “analysis of vegetational data” ((Tremp 2005) and “results of the biotope mapping in Baden-Württemberg” (Breunig & Höll 1995) helped to specify how to survey the parameters in the field.

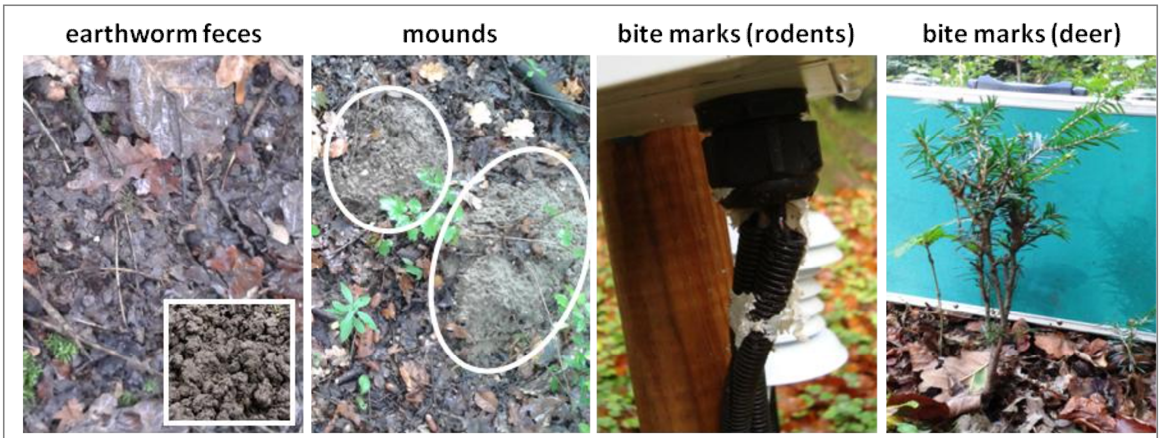
Table 3.4: Vegetation survey concept to characterize forest habitats according to a ticks needs.

	parameter	**Interconnection with ticks (grey field) / survey recording characteristics**
forest stand	**forest type**	Main habitat characteristic, indicator for the temporal variability of the protective function of the canopy (absorption of directly incoming solar radiation)
		Single coniferous trees in a broad-leaved stand, e.g., do not make a Mixed forest type. The character of the forest has to be considerably influenced by the other tree type, with a threshold level of 25%.
	canopy density	Degree of the protective function of the canopy
		Survey under full foliage **Low** – loose stand, high incidence of herbage and/or shrubs, wide and many sun spots at the ground when the sun shines **Medium** – characteristics in between low and high **High** – dense stand, low incidence of herbage and/or shrubs, , small and little sun spots at the ground when the sun shines
herbal layer	**herb layer density**	Efficiency of questing and protective function (dense herbage buffers microclimatic conditions better than loose herbage)
		Survey in summer and autumn **Low** – little and solitary herbs, floor dominated by leaf litter or the soil surface **Medium** – dense cover combined with large areas without plant cover or continuous but loose herbage, litter or soil visible **High** – dense cover with herbs, single and small areas without plant cover possible
	herb layer coverage	Accessibility of questing and protective function (ticks find shelter or quest on the vegetation only if the herbage is close enough)
		Continuous – high or medium herbage that is homogeniously spread over the area **Fragmentary** – herbage is clustered, wide areas without plant cover
litter layer	**litter depth**	Efficiency of protective function (ticks regain water losses if the humus is moist enough; “interception storage capacity”, Ch. 2.2.1.)
		Survey in late summer **Low** – only sparse leaf litter remains, mineral soil visible **Medium** – characteristics in between low and high **High** – several, thick layers of little to heavy decomposed litter
	litter coverage	Accessibility of protective function (ticks regain water losses if the moist litter is close enough)
		Continuous – high or medium litter that is homogeniously spread over the area, patches with lower or higher depth occur **Fragmentary** – the litter is frequently intermitted by litter free areas (due to e.g. fast decomposition, burrowing animals, wild boars)
	humus type	Main humus characteristic, indicator for the temporal variability of the protective function (as an outcome of this thesis)
	moss ground cover	alternative shelter when litter or herbage is missing

The resulting field check list for the vegetational survey can be found in Appendix (B2). Plants species and indicator plants were identified according to Jäger (2011) and Licht (2012) and the characteristica of the litter layer according to Sponagel (2005).

Only the **categorical parameters with the highest comparability** between study sites were used for analyses afterwards. Table 3.4 provides a detailed overview of parameters, their potential function for ticks and recording characteristics.

Beside parameters related to the vegetation, also qualitative signs were recorded related to humus and hosts of ticks (Figure 3.9), e.g., mounds of burrowing animals if they severly intermitted the humus layer and, thereby, disturbed the sheltering function of the litter and humus for ticks. One major assumption for this thesis is that the main **hosts of ticks**, such as small mammals and roe deer, are abundant in a suitable number at each study site. The fact that all study sites are forests, and most are broad-leaved or mixed forest types, lead to a certain assurance that the assumption is valid. However, distinct signs of hosts were recorded if existent.



fauna	*associated to humus*	**Earthworm feces**: sign for mull type humus and bioturbation **Mounds of burrowing animals**: intermit the humus layer
	associated to ticks (hosts)	Distinct signs for the occurrence of specific hosts: **Rodents**: bite marks at equipment, tunnels **Roe deer**: bite damages on young white fir are common if deer is frequent, damages on spruce indicate high deer abundances **Wild boar**: signs of rooting

Figure 3.9: Explanation and illustration of humus and host related parameters, recorded within the field survey.

Beside the pure identification of possibly relevant factors for ticks, another aim is to evaluate underlying processes (see Chapter 4.5). Doing this, the whole survey procedure could be reduced to relevant, easy to handle aspects of a factor. This is a **first step to provide a standard procedure** to characterize forest habitats of *I. ricinus* in the future, which would allow researchers to compare their results more easily. The need of comparable data in tick research was subject of a recently published paper (Estrada-Peña et al. 2013).

3.4 SOIL

The survey also included soil characteristics. The field and laboratory work as well as the analysis were executed by Benjamin Jonderko and Michael Wandler under the guidance of PD Dr. Stefan Norra, Martin Kull, Reiner Gebhardt and Denise Boehnke. Subsequent soil characteristics were examined at 17 study sites in total using soil profiles and core samples: soil type, horizon characteristics (boundaries, designation, sequence), texture, bulk density, skeleton content, mass of organic matter, actual humidity and chemical characteristics (pH value, lime content, electric conductivity) as well as the permeability (kf-value) and soil water tension (pF value). For further methodical details and results see Jonderko (2013) and Wandler (2013).

There is one important methodological restraint. The field work and the analysis of sample materials was done in the winter of 2012/2013. It was striking that only the humus types moder or raw humus were identified according to standardized documentary procedures following (Sponagel 2005) at all study sites, but no mull type humus (only mull-like moder). In contrast, the author identified several study sites with mull type humus in autumn 2014 using the same approach from Sponagel (2005). The season of exposure explains this discrepancy. In winter, the litter from the last defoliation still existed in full mass. This large mass was likely the major reason why the students identified no mull type humus, since an important characteristic is its fast decomposition and a low litter mass compared to the other types. Thus, to identify mull type humus it seems necessary to survey the matter at the end of the decomposition period (in late summer before the next leaf fall) or, even better, several times a year. Doing this, an additional procedure to estimate the decomposition rates could be carried out as well.

3.5 TICKS

Host seeking ticks were sampled using the common method of flagging (Estrada-Peña et al. 2013). Thus, ticks were collected monthly (March to October) by dragging a 1 m² cotton flag over 100 m² of woodland herbage, low shrubs and litter from summer 2012 to October 2015. Adult and nymphal ticks were removed every 10 m² and collected in separate vials for every drag. Larval ticks were removed after 100 m² flagging. Sampling took place only on dry days (no rain) at daytime.

Trained members of the *Zoological Institute, Karlsruher Institute of Technology*, sampled ticks at western study sides ("west": AH, AL, AW, FB, FR, HQ, HW, MB, PK, ST, SW) while trained personnel of the *Landesgesundheitsamt Baden-Württemberg* sampled at eastern sides ("east": BT, CW, DS, EP, FN, GH, KT, NA, PH, RF, VS, WP, WR, WU). All tick stages were identified to species level (Arthur 1963, Hillyard 1996, Pérez-Eid 2007), but only ticks of the species *Ixodes ricinus* were considered in this thesis.

At the sites PK and PH activity plots (as described in (Dautel et al. 2008) were installed in cooperation with Olaf Kahl and Hans Dautel, and tick collection was conducted more frequently per month. Therefore, monthly mean values per 100 m² were calculated for a better comparability with the other 23 sites.

I developed a special sampling design to reduce the impact of the flagging method on the number of sampled ticks and to make results from groups "West" and "East" more comparable. The preliminary study of this project (Boehnke 2011) showed that height of flagging has an influence on the chance of finding different tick stages in forest sites. Tälleklint-Eisen & Lane (2000) found that the type of vegetation strongly influence tick sampling results when ticks linger in lower parts of the vegetation and are therefore inaccessible with the flagging method. Therefore, three different height levels of dragging were defined: low (0-10 cm), medium (10-30 cm) and high (>30 cm). Monthly sampling on 100 m² is divided into: four drags on low level (40 m²), four drags on medium level (40 m²) and two drags on high level (20 m²). Additionally, other relevant factors were recorded, such as daytime, wind, radiation conditions (see Appendix (B3)).

In the following, the terms "total amount" and "abundance" are replaced by "density", which is generally used for the amount of ticks taken from a defined area size (Estrada-Peña et al. 2013). A useful term for the total amount of sampled ticks in one month is therefore the **monthly tick density.** Those of ticks sampled by monthly flagging over one season (March to October) on

100m² (following Schulz et al. 2014) is the (**annual**) **tick density** or (**annual**) **nymphal density** for exclusively the nymphal life stage.

The analyses of this thesis focus on nymphal data, since this life stage best represents the actual population densities when sampled by flagging (Milne 1943). Moreover, nymphs were found to be almost randomly distributed within a homogeneous habitat (Vassallo et al. 2000). The nymphal densities calculated by 10 samples can therefore be expected to be representative for each habitat. In addition, tick sampling data of the years 2013 and 2014 were used for analysis while data from 2015 were not included, because further funding for the data acquisition in 2015 was not conceivable at the time when the concept for the data analysis was designed in 2014.

3.6 CALCULATION OF MISSING DATA

Both the microclimatic and the tick raw data contain data gaps. As a consequence, calculation concepts were developed to replace the missing values for further analysis. The following chapter presents the models and calculation methods used in this thesis. Resulting values can be found in the next chapter, the appendix and on the data CD. Data gaps were filled for the years 2013 and 2014. Since the microclimatic and tick data sampling started mid-season 2012, no replacement could be calculated for the first half of the year.

3.6.1 CLIMATE DATA

When the batteries reach a low level and have to be changed, the sensor activity and recording have to be interrupted with a following restart. This leads to a short-time data gap of some 10-minute values. Those short-time gaps were replaced by means of surrounding values.

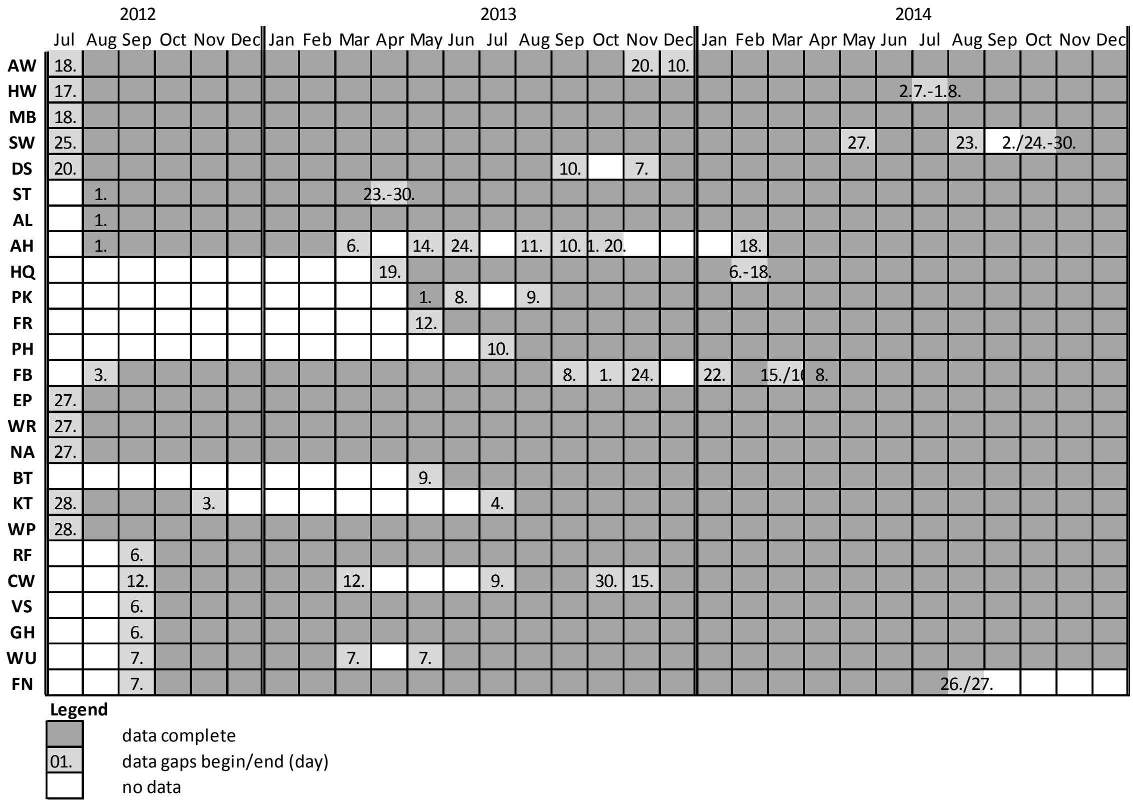
Not only short-time data gaps occurred but also long time gaps of several days to months due to technical issues. Table 3.5 gives an overview of the data situation for the years 2012 to 2014. For some calculations, for example to calculate the monthly or yearly means of parameters, it was necessary to have continuous data series. Therefore, data were replaced on the basis of daily averages using R version 3.0.1 (R Core Team 2013).

Processing

Daily averages of the parameters air temperature, soil temperature and relative humidity were calculated if the daily dataset was complete, based on data with 10-minute resolution. Data gaps were denoted as “NA” (using Excel 2007).

To choose the best substitution pairs, a cross-correlation analysis was performed between all sites using R (data status of Nov 2014). Only sites with non-overlapping data gaps were paired.

Table 3.5: Overview of the recorded data set of each study site during July 2012 and December 2014.



	2012						2013												2014											
	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
AW	18.	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	20.	10.	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
HW	17.	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	2.7.-1.8.	▨	▨	▨	▨	▨
MB	18.	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
SW	25.	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	27.	▨	▨	23.	2./	24.-30.	▨	▨
DS	20.	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	10.		7.	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
ST		1.	▨	▨	▨	▨	▨	▨	▨	23.-30.	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
AL		1.	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
AH		1.	▨	▨	▨	▨	▨	▨	6.		14.	24.		11.	10.	1. 20.				18.	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
HQ										19.	▨	▨	▨	▨	▨	▨	▨	▨	▨	6.-18.	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
PK											1.	8.		9.	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
FR											12.	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
PH													10.	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
FB		3.	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	8.	1.	24.		22.	▨	15./1	8.	▨	▨	▨	▨	▨	▨	▨	▨
EP	27.	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
WR	27.	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
NA	27.	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
BT											9.	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
KT	28.	▨	▨	▨	3.								4.	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
WP	28.	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
RF			6.	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
CW			12.	▨	▨	▨	▨	▨	12.				9.	▨	▨	30.	15.	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
VS			6.	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
GH			6.	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
WU			7.	▨	▨	▨	▨	▨	7.		7.	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
FN			7.	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	26./27.				

Legend

▨	data complete
01.	data gaps begin/end (day)
☐	no data

The models

Simple linear regression was performed for each parameter and site by using R (function lm(), with ordinary least-squares algorithm OLS). The input data range from 2012-07-18 to 2015-03-13 (status of July 2015). The model summary (Table 3.6) contains the following information: the response variable (gap-site = Y), the predictor variable (pred-site = X), the estimated factor which is the air-temperature (Ta), soil-temperature (Ts) or relative humidity (RH), the regression coefficients a and b for the estimated simple regression equation

$$y = a + b^{*}x,$$

the number of paired observations (n) used for the model, the coefficient of determination (R^2) as the measure for model quality, the p-values of the t-statistics which are a measure for the quality of the estimated coefficients a and b (if *p*-value is > 0.1 the likelyhood that the coefficient adds nothing to the model increases), the *p*-value of the F statistic which tests if the model itself is significant (true if < 0.1) and the residuals statistics (note that the OLS algorithm calculates residuals with a mean of zero). Programming and interpretation are processed using the literature of (Teetor & Loukides 2011).

Table 3.6: Statistics of the data gap substitution models.

			Coefficients				*Test statistics*				*Residuals statistics*			
Y	**X**	**factor**	**a**	**b (x)**	**n**	**R²**	**p -(a)**	**p (b)**	**p (F)**	**Min**	**qu_1**	**Med**	**qu_3**	**Max**
AW	ST	Ts	0,082	1,007	796	0,99	0,02	0	0	-1,4	-0,2	0,0	0,2	5,8
AW	ST	Ta	0,327	0,962	858	1,00	0	0	0	-2,4	-0,2	0,0	0,3	1,7
AW	ST	RH	10,899	0,875	858	0,82	0	0	0	-14,2	-2,1	0,0	1,7	20,7
ST	AW	Ts	-0,009	0,987	796	0,99	0,79	0	0	-5,6	-0,2	0,0	0,2	1,4
ST	AW	Ta	-0,286	1,034	858	1,00	0	0	0	-1,8	-0,3	0,0	0,2	2,5
ST	AW	RH	5,668	0,94	858	0,82	0	0	0	-24,2	-1,1	0,5	2,0	13,3
AH	SW	Ts	-1,778	1,058	623	0,99	0	0	0	-1,9	-0,2	0,0	0,2	2,0
AH	SW	Ta	-0,914	0,98	616	0,98	0	0	0	-2,7	-0,5	-0,1	0,5	5,3
AH	SW	RH	10,388	0,928	616	0,84	0	0	0	-24,2	-3,4	-0,7	3,7	19,8
SW	AH	Ts	1,737	0,938	623	0,99	0	0	0	-1,9	-0,2	0,0	0,2	1,7
SW	AH	Ta	1,084	1,001	616	0,98	0	0	0	-5,4	-0,4	0,1	0,5	2,7
SW	AH	RH	4,098	0,903	616	0,84	0	0	0	-20,3	-3,7	0,4	4,2	23,8
DS	WP	Ts	-0,958	1,073	864	0,99	0	0	0	-2,2	-0,4	0,0	0,3	2,3
DS	WP	Ta	0,034	0,975	863	0,98	0,5	0	0	-3,7	-0,6	-0,2	0,3	6,6
DS	WP	RH	0,908	0,962	863	0,80	0,54	0	0	-47,0	-2,0	1,0	3,2	10,8
WP	DS	Ts	1,006	0,918	864	0,99	0	0	0	-2,0	-0,3	0,0	0,3	2,0
WP	DS	Ta	0,122	1,005	863	0,98	0,02	0	0	-6,8	-0,3	0,2	0,6	3,5
WP	DS	RH	17,426	0,827	863	0,80	0	0	0	-13,5	-2,6	-0,2	2,3	38,5
HQ	ST	Ts	0,928	0,822	634	0,98	0	0	0	-1,5	-0,4	0,1	0,4	1,6
HQ	ST	Ta	0,009	0,913	634	0,99	0,83	0	0	-2,6	-0,3	0,0	0,3	1,7
HQ	ST	RH	8,376	0,939	634	0,84	0	0	0	-16,9	-1,9	-0,4	2,0	17,1
PK	ST	Ts	0,36	0,964	578	0,99	0	0	0	-1,1	-0,2	0,1	0,2	1,3
PK	ST	Ta	-0,151	0,999	534	1,00	0	0	0	-2,6	-0,2	0,0	0,2	1,3
PK	ST	RH	-12,766	1,137	534	0,93	0	0	0	-9,4	-1,1	-0,3	1,2	20,9
PH	NA	Ts	0,587	0,864	576	0,99	0	0	0	-1,5	-0,3	0,1	0,3	1,5
PH	NA	Ta	-1,318	0,985	576	0,99	0	0	0	-3,0	-0,5	-0,1	0,4	3,8
PH	NA	RH	-1,957	1,027	576	0,81	0,31	0	0	-18,4	-1,5	0,0	2,2	18,8
FB	FR	Ts	-4,471	0,913	564	0,94	0	0	0	-2,9	-0,7	0,1	0,7	3,5
FB	FR	Ta	-4,971	0,95	566	0,93	0	0	0	-5,9	-1,0	-0,1	0,7	10,3
FB	FR	RH	39,629	0,612	567	0,50	0	0	0	-37,8	-1,7	0,7	3,7	23,8
CW	WR	Ts	-0,357	1,026	740	1,00	0	0	0	-0,9	-0,2	0,0	0,2	1,2
CW	WR	Ta	0,05	0,969	758	0,99	0,2	0	0	-2,6	-0,3	0,0	0,4	3,1
CW	WR	RH	15,256	0,838	758	0,89	0	0	0	-13,3	-2,1	0,4	1,8	19,9
WU	FN	Ts	-2,264	1,189	659	0,97	0	0	0	-3,3	-0,8	0,0	0,7	2,7
WU	FN	Ta	-1,718	1,026	659	0,98	0	0	0	-6,3	-0,5	0,0	0,5	2,9
WU	FN	RH	8,126	0,889	659	0,70	0	0	0	-13,8	-3,1	0,7	3,0	12,8
FN	WU	Ts	2,145	0,816	659	0,97	0	0	0	-2,2	-0,6	0,1	0,6	2,5
FN	WU	Ta	1,797	0,959	659	0,98	0	0	0	-2,8	-0,5	0,0	0,5	5,8
FN	WU	RH	21,199	0,79	659	0,70	0	0	0	-18,3	-1,9	-0,1	2,4	11,9
HW	ST	Ts	0,799	0,909	904	0,99	0	0	0	-2,8	-0,3	0,1	0,4	1,4
HW	ST	Ta	0,016	1,001	904	1,00	0,56	0	0	-5,0	-0,2	0,0	0,2	1,7
HW	ST	RH	-16,582	1,148	904	0,87	0	0	0	-15,1	-2,4	0,2	2,0	20,6
FR	AL	Ts	-0,528	0,981	668	0,99	0	0	0	-1,2	-0,4	0,0	0,3	1,8
FR	AL	Ta	-0,528	0,981	668	0,99	0	0	0	-1,2	-0,4	0,0	0,3	1,8
FR	MB	RH	12,178	0,86	629	0,74	0	0	0	-45,8	-3,2	0,4	3,4	27,0
BT	NA	Ts	1,101	0,882	637	0,99	0	0	0	-1,6	-0,3	0,0	0,4	1,3
BT	NA	Ta	-0,321	1,004	637	0,98	0	0	0	-2,1	-0,5	-0,2	0,4	4,6
BT	NA	RH	-20,477	1,194	637	0,87	0	0	0	-20,6	-1,6	0,5	1,8	12,4
KT	NA	Ts	-0,893	1,03	680	1,00	0	0	0	-1,5	-0,2	0,0	0,2	1,5
KT	NA	Ta	-0,925	1,009	680	0,99	0	0	0	-5,0	-0,4	0,0	0,4	2,1
KT	NA	RH	13,051	0,87	680	0,83	0	0	0	-15,9	-1,4	0,1	1,7	13,1

In general, the model quality is very high for temperature (Ta/Ts) with a mean R^2 of 0.98 and to a lesser extent for the relative humidity (RH) with a mean R^2 of 0.80. The *p*-values for all coefficients of the predictor variable are smaller than 0.001 (highly significant ***), which indicates a good pairing of response and predictions sites. The regression residuals (see Table 3.6, Residuals statistics) should have a perfectly normal distribution. Looking at the median values (see Table 3.6, Med), 45% of the models show no skew (med = 0), 20% have a slight skew to the left (max = -0.7) and 35% a slight skew to the right (max = 0.7) which is very small considering the scope of the variables “temperature” and “relative humidity”.

The min and max show some extreme outliers in the residuals, especially for relative humidity, but also many models with a very good performance, which is not surprising concerning the models R^2 with a mean skew of 0.1.

The smaller the magnitude of first and third quartile of the residuals the better, since this value expresses the deviation of the true value and estimated value (= residual) for 50% of paired observations. The mean deviation is 0.1, which is very small considering the scope of the variables “temperature” and “relative humidity”. The min and max show some extreme outliers in the residuals, especially for relative humidity, but also many models with a very good performance (Ts/Ta: > -2 and < 2), which is not surprising looking at models R^2.

To minimize processing errors, the R source code for the linear regression models were printed out and checked visually and time series of the newly calculated “gap-sites” were plotted and checked visually for gaps or cracks. Since newly calculated daily means of the relative humidity sometimes surpass 100%, all values were capped to 100% afterwards using Excel 2007.

3.6.2 TICK DATA

Monthly flagging from March to October was not possible at each study site due to rain, snow cover, flooding or other issues. Since the tick density per year is the total sum of flagged ticks, missing values lead to an underestimation of the total tick amount compared to sites with a full data set.

To solve this problem, a calculation method to regain missing values was developed (in collaboration with Franz Rubel and Katharina Brugger, Institute for Veterinary Public Health, University of Veterinary Medicine Vienna, Veterinärplatz 1, A-1210 Vienna, Austria).

For each study site with a full dataset from March to October, the percentage proportion of nymphs per month is calculated. For example, site A had a total amount of 100 nymphs in 2013 with 25 nymphs in May - then the percentages proportion for May 2013 would be 25%. The mean of the percentage proportion is calculated for each month using all complete study sites. These percentages represent the proportion of a missing monthly value of the total amount of ticks in a year and were used to calculate missing nymphal densities at other sites. For example: Site B has a total amount of 150 nymphs for 2013 with a missing value in May. The amount of 150 nymphs in 2013 is therefore only 75% (100% - percentage in May (25%)) of the expected nymphal density. The calculated nymphal density for May 2013 is given as 150/75*25 → 50 nymphs, and the corrected nymphal density at site B for 2013 would be 200.

An overview of the monthly percentage proportions is given in Table 3.7.

Table 3.7: Percentage proportion of monthly nymphal densities in the course of the tick season 2013 and 2014, combining the results of all study sites.

year	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**
2013	3%	22%	23%	20%	18%	7%	5%	3%
2014	13%	18%	25%	16%	10%	4%	5%	8%

For data of the year 2013, calculations were made with the sites: AH, AL, AW, FR, HW, MB, PH, PK and ST (9 in total). For 2014: AH, AL, AW, FB, FR, HQ, HW, MB, PH, PK, ST and SW (12 in total). The complete datasets for monthly nymphal densities in 2013 and 2014 are given in Appendix (B4). The original (NymphDens) and corrected (NymphDens cor) annual nymphal densities are shown in the next Chapter in Table 4.1.

At the two sites Parzelle Karlsruhe (PK) and Parzelle Hohenheim (PH), a special setting containing defined numbers of tick individuals per parcel were installed to observe relative tick activity patterns throughout the year (Dautel et al. 2008). To combine these data with activity patterns of the actual tick populations at the study sites, flagging was usually done more than once per month. To get comparable data to the 23 other sites, doubled or tripled data were averaged to one value representing the monthly nymphal density. Averages are not calculated for larvae and adults.

3.7 STATISTICAL METHODS

All collected data on *Ixodes ricinus* ticks and microclimatic parameters were recorded in Microsoft Excel 2007. Monthly and annual numbers of sampled ticks and, in addition, hourly, daily and annual mean values of microclimatic parameters were also calculated in Excel. Afterwards, the dataset was analyzed using Excel 2007 or R version 3.0.1 (R Core Team 2013).

Techniques of **descriptive statistics** were used to get a first data overview. Such were: histograms, boxplots, line charts, run charts, network diagrams, scatter plot(s) (matrices), basic statistics (mean, max, min, standard deviation etc.).

3.7.1 HYPOTHESIS TESTS

Prior to statistical analysis, data were tested for normal distribution using the **Shapiro-Wilk-Normality test** and evaluated visually using histograms and quantile-quantile plots. Compared to other tests for normality, e.g. the Kolmogorov-Smirnov test or Chi-squared test, the Shapiro-Wilk test is most suitable for small sampling sizes. If the null hypothesis of a normal distribution was rejected, non-parametric tests were used (Kruskal-Wallis test, Wilcoxon signed-rank test, Mann-Whitney U Test, Spearman's rho). *P*-values smaller than 0.05 were characterized as significant, values smaller than 0.001 as highly significant.

To test if the total amount of sampled ticks of one population (= one study site) were significantly different in 2013 compared to 2014, the non-parametric **Wilcoxon signed-rank test** for related samples was used.

To test if the non-normal distributed densities of larvae, nymphs and adults differed significantly between the study sites, the **Kruskal-Wallis rank sum test** (or One-way ANOVA on ranks) for independent samples was performed based on the monthly tick data of each study per year.

The non-parametric **Mann-Whitney U Test** for independent observations was used to test if annual tick densities found at study sites higher 500 m a.s.l. (group 1) come from the same population as tick densities found at study sites lower 500 m a.s.l. (group 2).

Spearman's rho was used to evaluate the dependence between the nonnormal annual nymphal densities (corrected) and mean annual air / soil temperature and altitude, using the cor.test() function in R (R Core Team 2013). Litter temperature data was not included because of its too small sampling size (n = 5). The correlation coefficient (r_s) was calculated and tested for significance (*p*-value) using 1) all study sites (n = 25) or 2) study sites separated after altitude with sites higher (= high model, n = 11) and sites lower than 500 m a.s.l. (= low model, n = 13). In the low model, outlier with extraordinary high tick densities were removed (BT in 2013, AL in 2014). The non-parametric **Kruskal-Wallis test** was used to test for a significant influence of vegetation (categorical parameters, listed in Table 3.4) on nymphal densities (sum of nymphs per year and study site) following Teetor & Loukides (2011) and Dormann & Kühn (2011). Hereby it was checked if medians of nymphal densities between different groups (grouped after the vegetation parameter) are different (see Chapter 4.5.1). The null hypothesis of this test assumes that all groups (categorical variable) have the same median. If the null hypothesis is rejected ($p < 0.05$), two or more groups are very likely different. It is possible, that the test identifies a significant impact of a vegetational factor but this effect belongs to an altitudinal effect: If one vegetation parameter concentrate on high altitudes, the comparably low tick abundances are likely not caused by this vegetation parameter but rather by unsuitable temperature conditions. To evaluate this problem, all vegetation parameters building significantly different groups of nymphal densities were visualized afterwards, separated in study sites of low (< 500 m.a.s.l., Zone a), high altitude (> 500 m.a.s.l., Zone b) and the highest study site Feldberg (Zone c, outside of the tick distribution boundary).

In Gosheim, ticks were sampled in three different parts of a large forested area at nine dates between April and September 2014 (see Chapter 3.2.3). To test if the amount of sampled ticks were significantly different between different parts, the non-parametric **Kruskal-Wallis test** was performed using the results of the nine sampling dates, separated after life stages (larvae, nymphs and adults), and the sampling area (wide clearing, timberland, mixed stand) (Chapter 4.5.2.2).

3.7.2 SCATTER PLOT MATRICES

Scatter plot matrices visualize pairwise relationships among several variables. The lower triangle contains scatter graphs for each pair of variables and LOWESS smoothed lines (locally weighted Scatterplot smoothing, a non-parametric regression method), the upper triangle the calculated correlations coefficients and the diagonal depicts each variable and its data distribution via a histogram. The matrices were coded using R (R Core Team 2013) and the package *corrplot* (Wei 2013) following the instructions of (Chang 2013).

Matrices show the relationships between:

- the number of nymphs per year (nymphal densities corrected), altitude and annual averages of microclimatic variables (Chapter 4.4.1). Spearman’s rho was calculated, since tick data are not normally distributed. Outliers were removed for operating (BT in 2013, AL in 2014).
- the hourly averaged data of a microclimatic factor, such as soil, litter and air temperature and relative humidity, measured at different spots within the forest of an Intensive Site (Chapter 4.4.2). Pearson’s product-moment correlation coefficient was used to measure the linear correlation between each pair of variables.

In Chapter 4.4.1, relations between annual nymphal densities (corrected) and mean annual air temperatures as well as the parameter altitude are visualized using separate scatter plots for 2013 and 2014.

3.7.3 CLUSTER ANALYSIS

Cluster analysis is a form of explanatory data mining. Here it is used to group study sites together (cluster) which are more similar in respect to one or several parameters compared to other sites in other groups (clusters) (Bortz & Schuster 2010).

Groups of study sites with similar numbers of ticks were calculated on the basis of the total amount of sampled nymphs (corrected) seperatly for each year following (Teetor & Loukides 2011, Chang 2013). Firstly, the euclidean distances between all nymphal densities (n = 25) were computed using the method “mcquitty”. Secondly, hierarchical clusters were created and finally results were visualized by two cluster dendrograms, one for 2013 and one for 2014. For processing reasons, the study site “Neckaraue” (NA) was abbreviated with “HH. Computation was done using R (R Core Team 2013) and the package *cluster* (Maechler et al. 2015).

The same approach was used to identify different groups of sites on the basis of mean annual soil temperatures and nymphal density observations. To avoid false clustering due to different value ranges of nymphal densities (0 to several hundred) and mean annual temperatures (about 8°C+/-4) observations were standardized before using. As an example: (NymphDens14 - mean(NymphDens14))/sd(NymphDens14) with NymphDens14 as the total amount of sampled nymphs in 2014, its calculated mean and standard deviation (sd).

3.7.4 REGRESSION MODELS

To tests for a linear relationship between annual nymphal densities (corrected; = response variable) and mean annual soil and air temperatures as well as the altitude (= predictor variables) a **simple linear regression** was performed in R. The results contain: a measure of the model's quality (adjusted R²) which gives the fraction of the variance that is explained by the regression model and the *p*-value of the F-statistic, which indicates if the model is significant (Teetor & Loukides 2011). The same approach was used to tests for a **exponential** relationship by transforming the annual nymphal density data via the natural logarithm before using the lm() function in R.

Since the visual observation of the scatter plots indicate a relationship between nymphal densities and annual temperature data and altitude, respectively, only for study sites of higher altitudes, data were subdivided and tested again following Dormann & Kühn (2011) and Toms & Lesperance (2003). Linear regression models were operated with data subdivided in study sites higher (n = 11) and lower (n = 13, outlier removed – BT in 2013 and AL in 2014) than 500 m a.s.l., using a **piecewise linear regression**.

To test for a relationship between annual nymphal densities (corrected; = response variable) and the category variables forest type (B = broad-leaved/deciduous, C = coniferous, M = mixed, A = others) a **multiple linear regression** model was performed following (Dormann and . Results contain the estimated regression coefficients β, the standard error SE and the test statistic for each regression coefficient given as t-statistics t and the *p*-value. In addition, the models quality measure (adjusted R²) and its significance (*p*-value) are given. The same approach was used again on data subdivided in study sites higher and lower 500 m a.s.l.. Results of the alternative analysis of variance (ANOVA) were not included in further analysis since the assumption of a normal distribution of the resulting model residuals was not provided (tested with Shapiro test).

3.7.5 BACKWARD STEPWISE REGRESSION

One way to select environmental factors that have an influence on annual tick densities (sum of questing ticks in a year) is to put in all factors (full model) and remove factors with a weak impact step by step (reduced model) – a *backward stepwise regression* (Chapter 4.4.1).

Tested explanatory variables are mean annual T_A, T_S, RH, SD (2013 and 2014), mean spring T_A, T_S, RH, SD (March, April and May 2013 and 2014) and altitude. Additionally, the duration of vegetation period in days was included for 2014. The dependent variable is the corrected annual nymphal density. A comparison of results based on original versus corrected tick data shows no (2014) or negligible (2013) differences, therefore the corrected data were used to minimize method related distortion. The data set contains all study sites (n = 25) or is reduced by outliers of extraordinary high nymphal densities (2013: n = 22-24, ouliers are BT or BT, ST and FN; 2014: n = 24, outlier is AL).

Mean annual values represent subordinated processes that lead to a specific microclimatic habitat suitability for ticks. Spring related values integrate the high diversity of weather occurrences, which could have a main impact on tick activity and, resulting, tick density, since the activity has a great impact on the chance of finding a host and that in turn lead to higher or lower mortalities. Statistics were carried out using R (R Core Team 2013), based on (Teetor & Loukides 2011).

3.8 TICK SPECIFIC ANALYSIS

3.8.1 PREDICTION OF TICK MORTALITY IN WINTER

The general decrease of tick abundances with growing altitude could be a consequence of conditions in winter. To evaluate this hypothesis, three possible phenomena causing tick mortality in winter are considered based on the former research results:

a) long periods of temperatures < -10°C (Dautel & Knülle 1997, Dantas-Torres et al. 2013)
b) frequent diurnal temperature variations from -5 to +10°C (Herrmann & Gern 2013)
c) ice contact due to frost occurrences in the litter and upper soil layer (Dautel 2010)

Data were examined from October 2013 to April 2014 using Excel 2007 (see Chapter 4.3.2).

Long periods of cold temperatures: First, it was checked if weather temperatures < -10°C occur in the soil or air temperature dataset (= raw data with 10 minute resolution). Since the soil temperatures never fell below 0°C and the air temperatures for most study sites never reached -10°C, no further analysis was done. Minimum air temperature values were visualized as Table.

To test for **frequent diurnal temperature variations**, daily minimum and maximum values of the air temperature data (raw data) were extracted over the entire wintertime. Soil and litter data are not included since the absolute minimum temperatures stayed above 0° and -2.3° C, respectively. Thereafter, these data were analyzed for days with $T_{MAX} > 9.9$°C and $T_{MIN} < -4.9$°C.

Another phenomenon causing tick mortality is **direct ice contact**. Thus, frost occurrence in the upper soil or within the litter layer can lead to an increasing mortality risk for ticks, if humidity is high enough to support the formation of ice. This freezing is called “soil frost” and occurs if the air layer located directly on the ground surface (up to 5 cm above the ground) cools down below the freezing temperature while the soil can, but doesn`t have to be frozen (www.dwd.de). To check if such frost was a relevant mortality factor in the winter 2013/2014, data from the litter layer (T_L, measured about 1 – 5 cm above the ground) were checked for temperatures < 0°C.

3.8.2 PREDICTION OF DEVELOPMENT RATES

One successfully used approach to estimate the species distribution boundaries of *Ixodes ricinus* in northern countries like Sweden is based on the duration of the vegetation period. I calculated the periods for 2013 and 2014 and found a good conformity of the given threshold in Sweden (170 days, Jaenson et al. 2009, 2011) with our values at the highest study sites Feldberg (no ticks and vegetation period below the given threshold) and Gosheim (ticks frequently found, vegetation period within the threshold range).

However, the vegetation period does not represent the actual temperature conditions within the period of vegetation, since it is just a summation term of days with conditions over a certain threshold (compare Chapter 3.2.5.4). This approach is therefore unsuitable to characterize the influence of the temperature factor on the tick’s living processes within their time of activity. Randolph (2002) used a more tick-related concept to estimate the effect of temperature on “seasonal population dynamics” based on development equations by Campbell (1948). She calculated the development rates of different tick stages, the end of development and correlated those with activity peaks from *I. ricinus* over a series of years.

In this new approach, I combined the informative value of the latter concepts: I used development rates to estimate the “temperature-dependent suitability” of our 25 habitats and to evaluate this method for its prediction power of the species distribution boundary. The analysis focus on the egg-to-larvae development, since a successful development of eggs is the basis for a stable tick population.

Temperature-dependent mean daily development rates (Y) for *Ixodes ricinus* eggs-to-larvae were calculated using the equations by Campbell (1948), given in (Randolph et al. 2002):

$$Y = - 0.0000112X^2 + 0.002305X - 0.0185 \quad (r = 0.997)$$

The mean daily development rates (Y) were calculated as the mean values of 24 hourly development rates per day, which are calculated using mean hourly temperature values (X, in °C) within the equation. The development starts after a threshold temperature of about 8.3°C is exceeded and grows exponentially afterwards.

Development rates are used in Chapter 4.3.3 to examine the variety of development conditions provided at all study sites and in Chapter 4.5.2 to examine its variety at different spots within one study site.

Chapter 4.3.3: **Daily development rates (DdR)** were calculated using soil temperature data (depth of 5 cm) with 10-minute resolution. Compared to the air, soil temperature data provide better estimations of conditions eggs are exposed to since female ticks lay their eggs in the shelter of the litter layer or on the ground. **Development rates per week** were calculated to illustrate the seasonal development over the year according to (Randolph et al. 2002).

Furthermore, I invented a concept for calculating other related information relevant to compare study sites and get new insights into the temporal aspect of tick development.

These "**developmental indices**" are:

- the date of initial development onset in spring
- the date when summation of DdR reaches 0.5 (50%)
- the date when summation of DdR reaches 1 (100% = egg development is completed)
- total sum of DdR in one year and the date when development ends.

The indices characterize key points of temperature-dependent development processes for eggs within one year and among the years 2013 and 2014 and are visualized as Table.

However, there are two essential restrictions: Firstly, the date of the key points is only realistic for eggs whose development processes initially start in spring of the same year. Thus, for eggs laid in winter or at the end of the season in the last year and, to be very precise, eggs that never experienced enough heat in winter to proceed their development. Secondly, the end of development (DdR = 1) does not automatically represent the time when eggs actually hatch. The egg stage is able to delay the end of development via an egg diapause (see Chapter 2.1.3) to achieve more suitable conditions for the sensitive phase of hardening after hatching.

Another way of getting into the data is to calculate the **last date** laid eggs would still be able **to complete their development** until winter prohibits further physiological processes. Therefore, the time of last egg laying for female *Ixodes ricinus* is calculated backwards from the development index “date when development ends” (DdR = 1) to the start of development (DdR = 0).

Chapter 4.5.2: The index “egg development is completed (DdR = 1)” is used to compare development conditions provided in the litter layer at different spots of a medium-dense mixed forest (see “Special measurement concept”, Chapter 3.2.3). In contrast to the latter calculations, it was not possible to calculate the actual date of development start in spring. Measurements started at 2014-04-01, which is the timely onset for calculations. However, the actual start was earlier relating to data from the Basic Station.

4 RESULTS AND DISCUSSION

4.1 TICK DENSITY PATTERNS

The thesis focuses on the distribution of *Ixodes ricinus* in Baden-Württemberg and underlying processes. As a first step, this chapter provides an overview on tick density and seasonal activity as well as biotic and abiotic conditions found at the 25 study sites. Parts of this chapter have already been published (Boehnke et al. 2015, Petney et al. 2013-2015).

4.1.1 SPATIAL PATTERN: TICKS AND STUDY SITES

Basic characteristics of the study sites and results of the 2-year monitoring of host-seeking nymphs as well as microclimatic conditions are given in Table 4.1 and Table 4.2.

Table 4.1 shows the names of the 25 study sites with abbreviations used in this thesis, for instance, along with observed nymphal densities (amount of nymphs sampled in a year on 100m²) of *Ixodes ricinus* in the uncorrected and corrected version for 2013 and 2014 (see Chapter 3.1 and 3.6.2).

Selected study sites reflect almost the entire altitude range of Baden-Württemberg with Auwald as the lowest (111 m a.s.l.) and Feldberg as the highest (1288 m a.s.l.) study site.

Questing ticks were collected at 24 of 25 study sites. No ticks were found at the highest study site Feldberg. The other 24 locations varied considerably in the total numbers of *I. ricinus* ticks. For example, at Kirchheim unter Teck only 8 / 5% (2013/2014) of nymphs were found compared to Botnang, which is located nearby on a similar altitude. Looking at study sites with low tick densities, ticks were recorded very rarely (< 10 nymphs/year/100 m²) only at Allerheiligen. All other sites showed a higher annual variability. A more detailed insight will be given in Chapter 4.1.2.3.

The numbers of ticks show a distinct decrease from the youngest (larvae) to the eldest development stage (adults): annual densities ranges from 0 to 9006 (larvae), 0 to 857 (nymphs) and 0 to 65 (adults) individuals in 2013 and from 0 to 637 (larvae), 0 to 374 (nymphs) and 0 to 157 (adults) individuals in 2014.

Monthly and annual data for larvae and adults are depicted in Appendix (C1). In addition, tick data of single drags (one sample on10 m²) are illustrated using Boxplots in Appendix (C2).

Table 4.1: Names and associated abbreviations (abbr.) of all study sites with geographical location (lon = longitude, lat = latitude), altitude in meter above sea level (m a.s.l.), ecological landscape (ecol. lands.: AV = Alpenvorland, NT = Neckar-Tauber-Gäuplatten, OT = Oberrheintiefland, SA = Schwäbische Alb, SK = Schwäbisches Keuper-Lias-Land, SW = Schwarzwald; X/Y – site is located in a transition zone between X and Y), forest type (C = coniferous forest, B = deciduous forest, M = mixed forest, A = Other), sum of collected nymphs (NymphsDens) and corrected sum (NymphsDens cor) for 2013 and 1014. Sites are ordered by altitude.

	abbr.	**name**	**lon**	**lat**	**altitude [m.a.s.l.]**	**ecol. landsc.**	**forest type**	**NymphDens**		**NymphDens cor**	
								2013	**2014**	**2013**	**2014**
1	**FB**	Feldberg	8,0382	47,8623	1288	SW	C	0	0	0	0
2	**GH**	Gosheim	8,7600	48,0999	992	SA	M	32	25	43	29
3	**AH**	Allerheiligen	8,1814	48,5337	773	SW	C	3	4	3	4
4	**DS**	Drackenstein	9,6562	48,5566	755	SA	B	47	50	63	82
5	**VS**	Villingen-Schwenningen	8,5666	48,0757	700	NT/SK	C	8	37	10	43
6	**WU**	Wurzacher Ried	9,9253	47,9390	665	AV	A	1	39	2	45
7	**WP**	Wippingen	9,8400	48,4153	659	SA	B	140	19	188	22
8	**SW**	Gaistal, Schwarzwald	8,4425	48,7740	610	SW	M	70	166	72	166
9	**CW**	Calw	8,8278	48,7419	532	NT	M	110	59	142	68
10	**RF**	Rosenfeld	8,7241	48,3219	512	SK	A	94	63	127	73
11	**WR**	Wüstenrot	9,4344	49,0994	504	SK	C	79	40	102	46
12	**PH**	Parzelle Hohenheim	9,0898	48,6801	472	SK	M	53	25	53	25
13	**FN**	Friedrichshafen	9,5121	47,6515	425	AV	M	369	170	496	196
14	**FR**	Freiburg	7,8816	47,9962	382	OT/SW	B	264	121	264	121
15	**BT**	Botnang	9,1300	48,7877	349	SK	M	857	147	1066	169
16	**KT**	Kirchheim Teck	9,4284	48,6286	337	SK	B	72	7	131	8
17	**EP**	Eppingen	8,9260	49,1107	268	NT	B	122	38	158	44
18	**MB**	Michaelsberg	8,5726	49,0882	253	OT/NT	B	259	167	259	167
19	**HQ**	Hedwigsquelle	8,4356	48,9545	215	OT/SW	B	341	245	351	245
20	**NA**	Neckaraue	9,2060	48,9862	204	NT	B	107	63	138	73
21	**AL**	Altenheim	7,7796	48,4693	147	OT	B	263	374	263	374
22	**PK**	Parzelle Karlsruhe	8,4257	49,0280	126	OT	M	101	46	101	46
23	**ST**	Staffort	8,5115	49,0763	122	OT	B	689	187	689	187
24	**HW**	Hardtwald	8,4798	49,1340	117	OT	M	297	230	297	230
25	**AW**	Auwald	8,3765	49,1338	111	OT	B	226	163	226	163

Climatic characteristics based on data from Basic Stations and averaged values of Intensive Sites are given in Table 4.2.

A mean temperature difference of more than 1 K was found between both years. The year 2014 was warmer, mean annual air temperatures ranging from 5.9° to 11.8°C (4.5° to 10.5°C in 2013) and soil temperature ranging from 6° to 12.4°C (4.7° to 11.1°C in 2013). Relative humidity and saturation deficit showed less difference between both years (RH: 84 to 94% in 2013; 81 to 94% in 2014 and SD: 0.7 to 2.5 hPa in 2013 and 0.8 to 2.8 hPa in 2014). The soil moisture ranges between 9 and 52% in 2013 and 6 and 53% in 2014. As expected, the highest values were measured on the swamp (WU). Lowest values were measured at a study sites with sandy soil, Hardtwald. The soil moisture was on average 8.5% higher in the year 2014 than in 2013. Only eight sites had higher annual soil moisture values in 2013, with five sites located in or near the *Oberrheintiefland*. The duration of the vegetation period ranges from 145/170 (Feldberg) days at minimum to 223/290 (Altenheim) days at maximum in 2013/2014.

Table 4.2: Mean annual values of the measured basic microclimatic parameters air (T_A) and soil (T_S) temperature in °C, relative humidity (RH) in % , soil moisture (SM) with m^3_{water}/ m^3_{soil} in % and the calculated saturation deficit (from daily means of RH and T_A) in hPa for 2013 and 2014. The vegetation period (VegPer) is given in days per year.

	T_A		**RH**		**SD**		**T_S**		**SM**		**VegPer**	
	2013	**2014**	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**
FB	4.5	5.9	95	91	0.7	1.1	4.7	6.0	0.34	0.37	145	170
GH	6.0	7.5	91	91	1.4	1.2	7.1	8.2	0.27	0.20	180	220
AH	7.4	9.2	88	86	1.8	2.1	7.3	8.8	0.27	0.30	205	260
DS	7.1	8.9	87	86	1.9	1.9	7.7	9.2	0.44	0.45	205	250
VS	7.1	8.4	95	94	0.8	0.8	8.2	9.1	0.27	0.30	210	235
WU	7.1	8.5	90	90	1.3	1.3	8.7	9.8	0.52	0.53	210	235
WP	7.4	9.1	89	88	1.6	1.8	8.1	9.3	0.23	0.24	210	250
SW	8.4	10.4	84	81	2.5	2.8	8.6	10.0	0.16	0.15	205	260
CW	8.0	9.9	89	87	1.6	2.0	8.5	9.7	0.17	0.21	210	260
RF	8.0	9.2	91	90	1.4	1.4	9.0	9.9	0.19	0.24	205	250
WR	8.2	10.1	88	86	2.0	2.2	8.6	9.8	0.22	0.24	210	260
PH	8.0	9.3	91	91	1.2	1.2	9.1	9.9	0.26	0.29	210	250
FN	8.6	9.9	92	92	1.1	1.1	9.3	10.0	0.24	0.24	210	260
FR	10.5	11.5	85	85	2.4	2.4	10.5	11.6	0.16	0.16	215	285
BT	9.1	10.6	87	88	1.8	1.8	9.7	10.6	0.19	0.17	210	270
KT	8.6	10.0	91	92	1.2	1.1	9.1	10.2	0.26	0.31	210	270
EP	9.3	10.9	91	91	1.4	1.4	9.8	11.0	0.24	0.24	210	270
MB	9.8	11.4	85	84	2.4	2.6	9.9	11.3	0.38	0.34	210	285
HQ	9.2	10.8	93	91	0.9	1.2	9.6	10.7	0.21	0.34	210	270
NA	9.3	10.9	90	90	1.3	1.5	9.7	10.9	0.26	0.29	210	270
AL	10.2	11.7	88	88	1.8	1.9	11.1	12.4	0.19	0.16	223	290
PK	9.8	11.6	90	89	1.6	1.8	10.3	11.7	0.26	0.29	210	285
ST	10.0	11.8	90	89	1.6	1.7	10.6	11.9	0.18	0.16	210	260
HW	10.1	11.8	88	84	1.8	2.6	10.4	11.8	0.09	0.06	220	270
AW	10.1	11.7	90	88	1.4	1.9	10.8	12.2	0.30	0.27	220	285

Descriptive statistics of the four measured parameters T_A, T_S, RH and SM are visualized as Boxplots in Appendix (C3). In addition, the variability of the parameters T_A, T_S, RH measured at different spots at the Intensive Stations are summarized as descriptive statistics in Tables in Appendix (C4). A list of the main plant species found at each study site is given in Appendix (C5).

4.1.2 TEMPORAL PATTERN: 2013 VERSUS 2014

This chapter contains a comparison of the results in 2013 and 2014. The climatic conditions of both years are characterized, along with tick densities and activity. The years 2012 and 2015 are excluded from analysis since (currently) no full annual dataset exists.

It has to be mentioned that parts of this chapter have already been published by the author in project reports (Petney et al. 2013-2015) and in Boehnke et al. 2015. Some Figures and Tables are either direct references or modified, whereas others are newly included to provide additional information.

4.1.2.1 CLIMATE

Figure 4.1 illustrates the monthly temperature averages for 2013 and 2014 compared to the long-term average (30 years from 1985-2014; data from DWD) for the region of Baden-Württemberg.

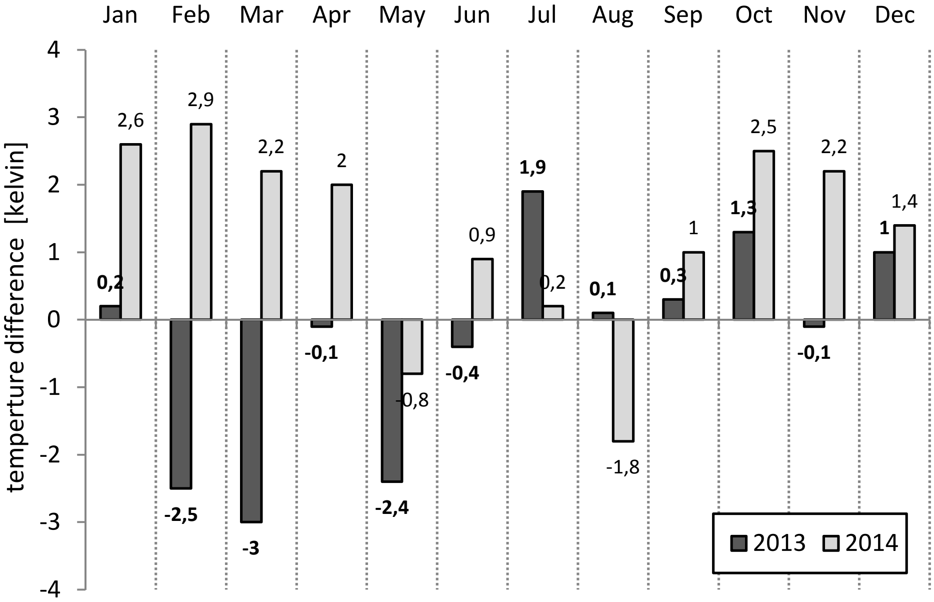


Figure 4.1: Comparison of the monthly mean temperatures in the years 2013 & 2014 with the 30-year average of the monthly mean temperatures (1985-2014); Data from " Zeitreihen für Gebietsmittel (time series for area means)," Land (area) Baden-Württemberg , German Weather Service, www.dwd.de; access on 13th January 2015)

Both years show a strong difference in their temperature development in the course of the year. In 2013, the first warm phase occurred in the middle of April. The spring season started late and was the coldest in the last 25 years with strong frost and snowfall in March and heavy rainfall in May.

The starting point of "full spring" was at 2013-05-02 (marked by the occurrence of the apple bloom, data from DWD). In contrast, the spring season of 2014 was very warm with 2.1°C above the 30-year average for March and April and "full spring" starting at 2014-04-07, almost one month earlier than 2013. For both years, the precipitation rate was too low in March and April and higher than usual in May, compared to the long time average.

The character of the summer 2013 was generally warm, rich in sunshine and rather dry, with frequently occurring storms and heavy precipitation events. In 2014, the summer was warm and dry, except for August. This month was cold and wet with heavy precipitation events and less hours of sunshine. The winter 2013/2014 was very mild (fourth warmest since beginning of regular measurements) and comparably dry while conditions in winter 2012/2013 were wetter and colder with extreme cold conditions in March (for example DWD 2014).

4.1.2.2 TICK PHENOLOGY

The following part presents results of the phenology of tick activity in both years. Data basis is the amount of monthly flagged ticks from March to October.

This part aims to

(1) give an overview of the activity situation in both years over all study sites
(2) compare the activity start in spring based on the first nymphal activity peak
(3) identify distinct monomodal and bimodal activity pattern

An overview of the entire nymphal activity situation in 2013 and 2014 is given in Figure 4.2, based on corrected nymph data. It can be seen that after their activation in spring, the main numbers of nymphs were active over 4 months, from April until July in 2013. In contrast, nymphs in 2014 were mainly active only in April and May and stayed at a low activity level afterwards. In the first month of flagging, March, ticks were more active in 2014.

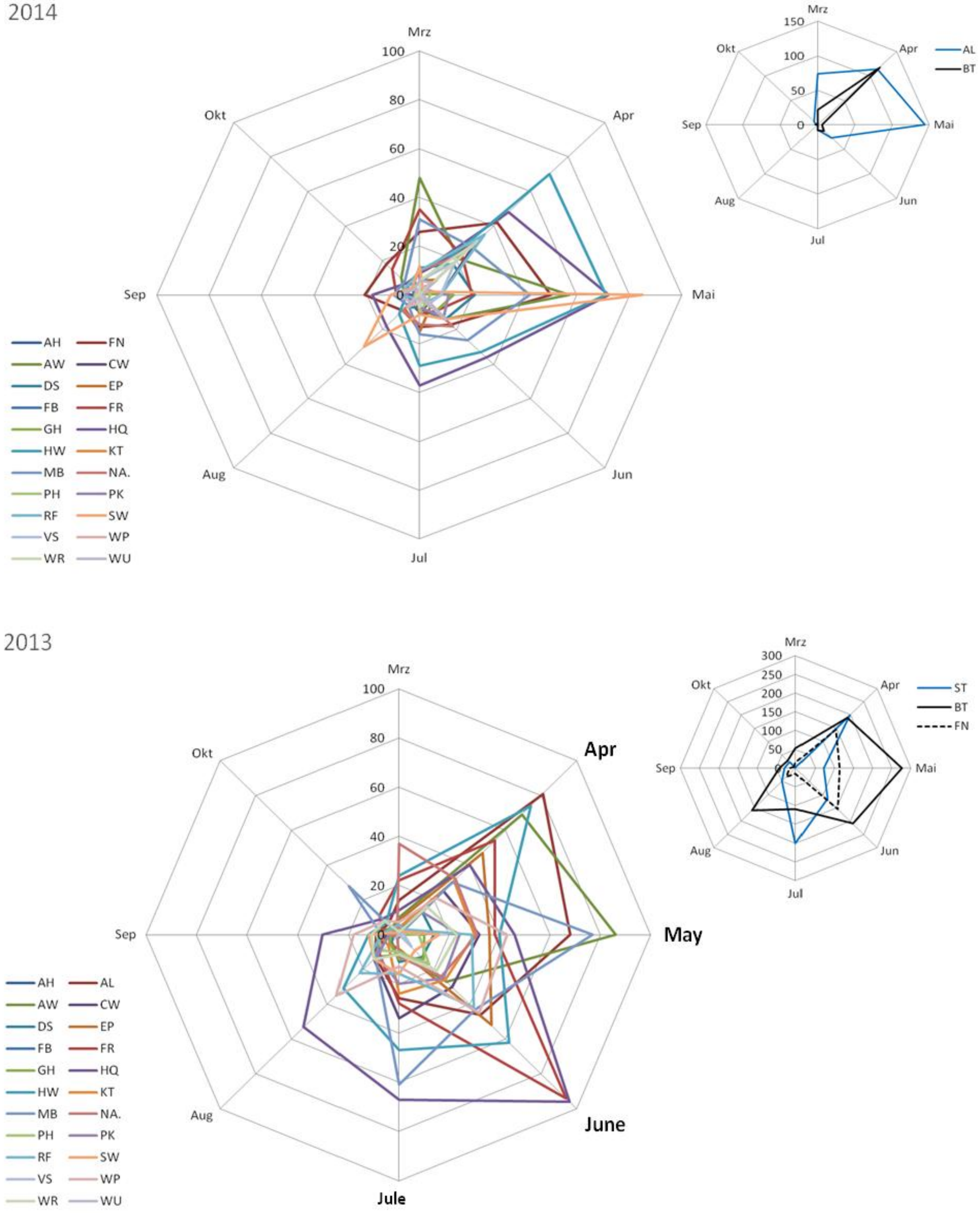


Figure 4.2: Tick activity at each site from March to October in 2013 (top) and 2014 (bottom), illustrated by the monthly nymph density (corrected). Study sites with more than 100 nymphs/month are shown separately (figures on the right). For 2013 this is ST, BT and FN. In 2014, high nymphal densities occur again in BT and, in addition, in AL.

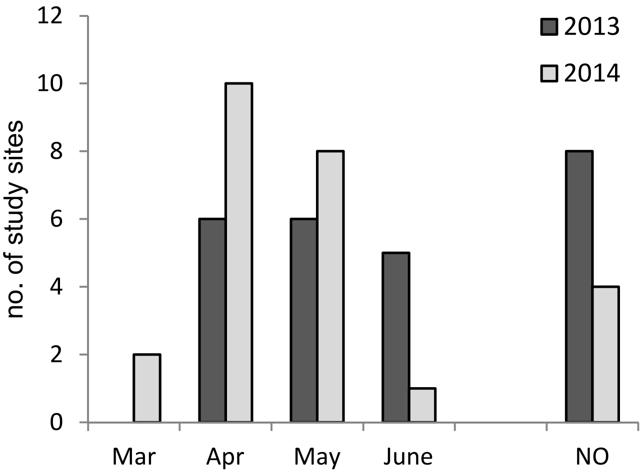
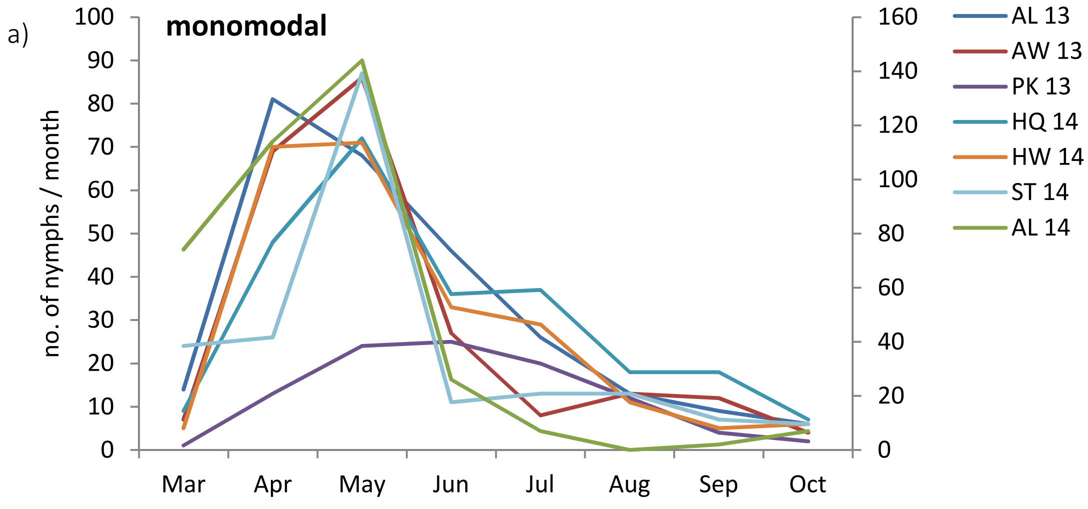


Figure 4.3: Occurrence of distinct *Ixodes ricinus* nymphal spring peaks in 2013 and 2014. Sites with very low or similar densities throughout the spring time were not counted (NO).

Figure 4.3 visualizes the first nymphal peak occurrence of all study sites. Line charts from monthly nymph data (not shown; same type as Figure 4.4) were the basis to identify the first peak visually. Only sites with a clear peak were taken into account. The nymphal activity started very early in 2014 as a result of the early start of an extraordinary warm springtime. Up to May 2014, the first activity peak was noticeable in 80% of the study sites.

In the very cold springtime of 2013, a clear peak was only noticeable at 48% of all sites until May and a higher proportion of sites (32% in 2013, 20% in 2014) showed no clear peak at all. Five of the study sites then showed a late peak in June 2013 whereas no peak was identifiable in June 2014. Sites with a first nymphal peak in June 2013 have altitudes above 450 m a.s.l. (PH, RF, VS, GH) or a specific cool climate (the ravine habitat HQ).

Figure 4.4 illustrates monomodal and bimodal acticity pattern from March to October 2013 and 2014. Only study sites with a full tick dataset were taken into account, since the visual characterization of activity patterns would be distorted by missing monthly values. Therefore, a total of nine sites were evaluated in 2013 and eleven sites in 2014. Distinct monomodal pattern of nymphal activity were observed at three sites in 2013 and four sites in 2014. Bimodal activity patterns occurred at two sites in 2013 and three sites 2014. In addition, indistinct activity patterns were observed at AH, FR, HW and PH in 2013 and at AH, FB, MB, PH and PK in 2014. Only the study site Altenheim (AL) showed a consistent monomodal activity pattern in both years. The rest of the evaluated study sites showed a mixture of monomodal, bimodal and indistinct activity patterns throughout the tick seasons of 2013 and 2014.



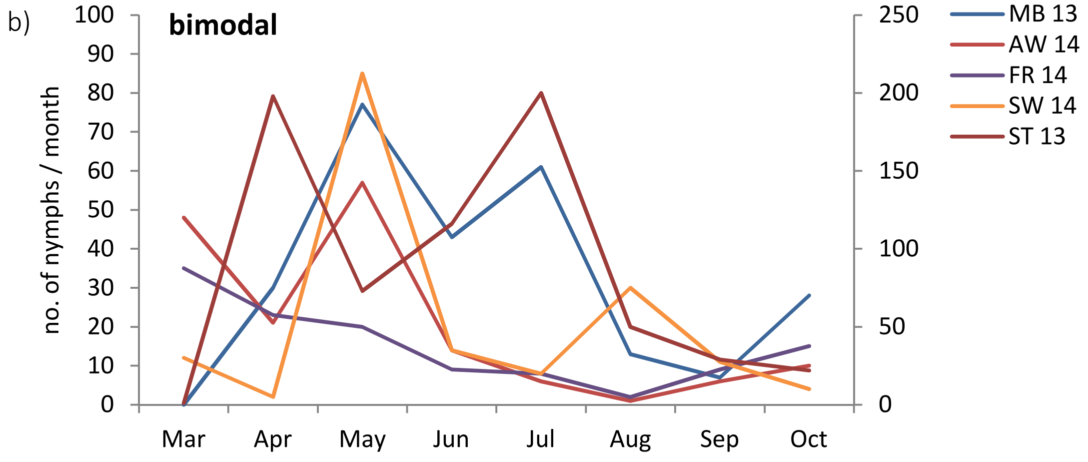


Figure 4.4: Observed monomodal (a) and bimodal (b) nymphal activity pattern in 2013 and 2014. The legend labels study site and year. Sites with high nymphal densities are plotted on the second value axis (AL 14 in a) and ST 13 in b)) for illustration reasons.

4.1.2.3 TICK DENSITY

The following Chapter compares annual densities of questing ticks collected in 2013 and 2014.

This part aims to

1. present the total annual amount of collected *I. ricinus* ticks separated after development stage
2. give an overview of the tick density situation at all study sites and of all life stages
3. identify groups of comparable annual nymphal densities.

From March to October 2013 and 2014, a total of 22,697 *I. ricinus* ticks were collected over the 25 study sites (Figure 4.5). The total amount of larvae and nymphs was reduced by 76% and 46%, respectively, from 2013 to 2014. Only the adult stage showed an increase of 36%.

The graphics of Figure 4.6 contain information about two major aspects: the **intra-variability** of annual tick densities at one study site within the years 2013 and 2014 and the **inter-variability** of the differences among the study sites.

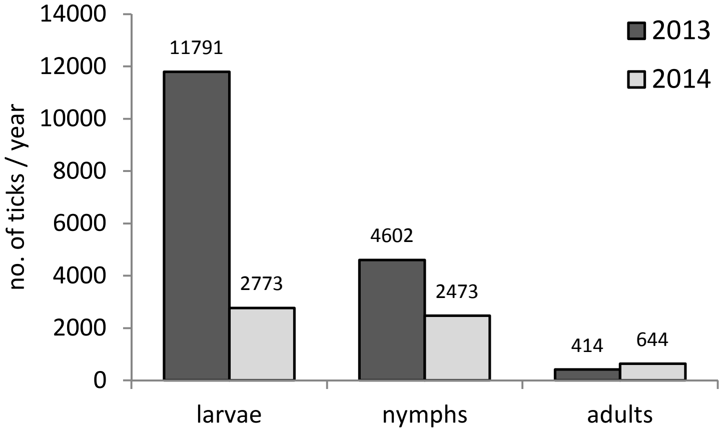


Figure 4.5: Total abundance of host-seeking ticks sampled in 2013 and 2014.

Looking at the annual tick densities in 2014 compared to 2013, the **intra-variability** of nymphs ranges from 10% to 3900% (Figure 4.6a), of larvae from 0% to 867% (Figure 4.6b), and of adults from 46% to 620% (Figure 4.6c). Nymphal densities decreased distinctively from 2013 to 2014 at most study sites, larvae to a lesser extent (larvae: 13 sites; nymphs: 18 sites). According to the **Wilcoxon signed-rank test** nymphal and adult densities differed significantly ($P < 0.05$) between the years.

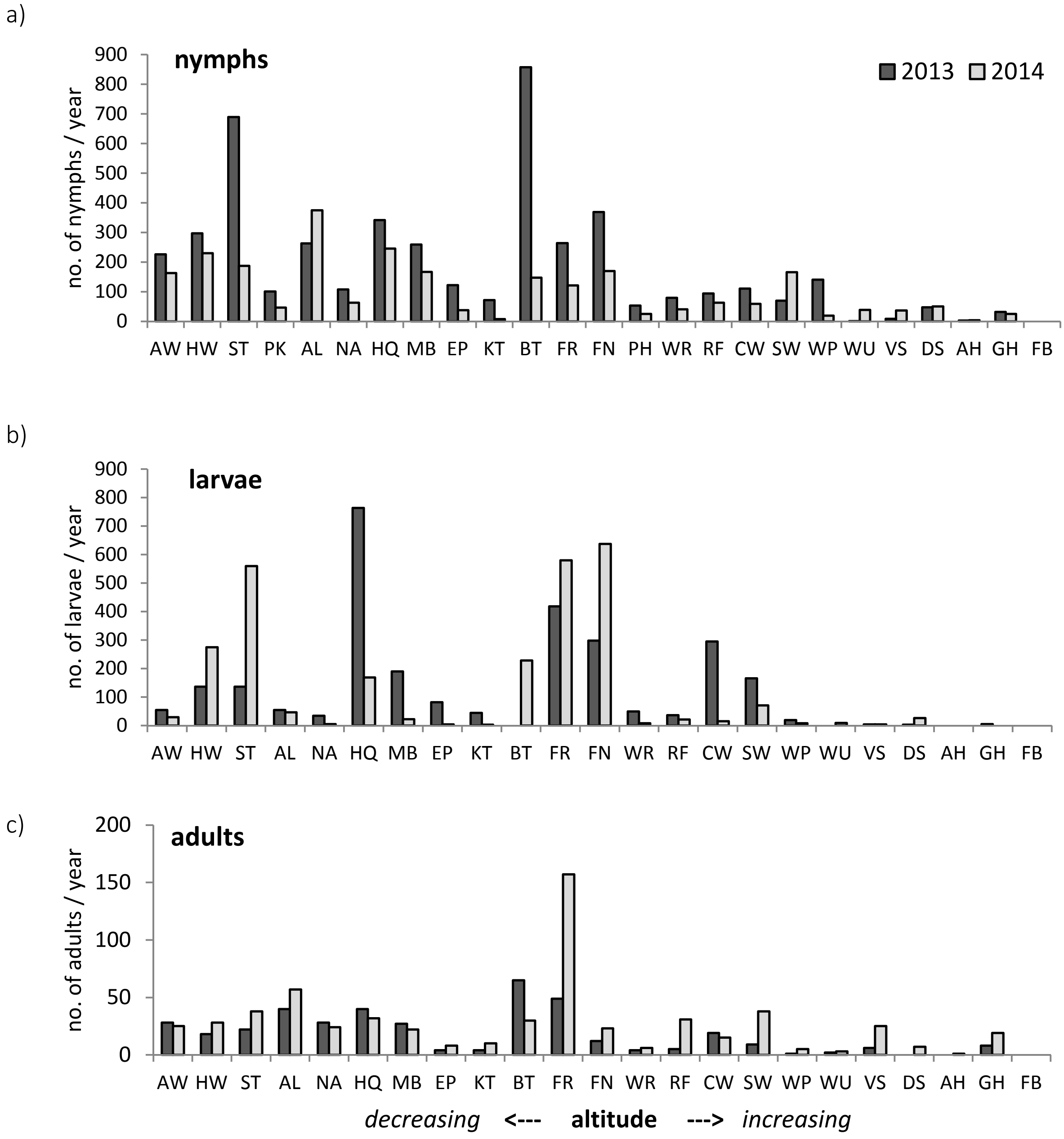


Figure 4.6: Total amount of a) nymphs, b) larvae and c) adults found in 2013 and 2014 at each study site. Sites are ordered by altitude (AW – lowest, FB – highest, in m a.s.l.). The number of larvae in BT 2013 (9006 l.) are not shown in figure b) due to its high extent for illustration reasons.

Interestingly, an increase of nymphal densities from 2013 to 2014 was mainly observed at study sites in areas of higher altitude - with 610 to 755 m a.s.l. (VS, WU, SW, DS) except for one lowland forest (AL). In conclusion, the study sites show a high variability in annual tick densities within the examined time span.

The **inter-variability** of annual tick densities shows two trends: Tick densities are constantly low at higher altitudes (right part of the histograms) and, secondly, tick densities show a remarkable, non-altitude dependent variability at low and medium altitude (left & middle part).

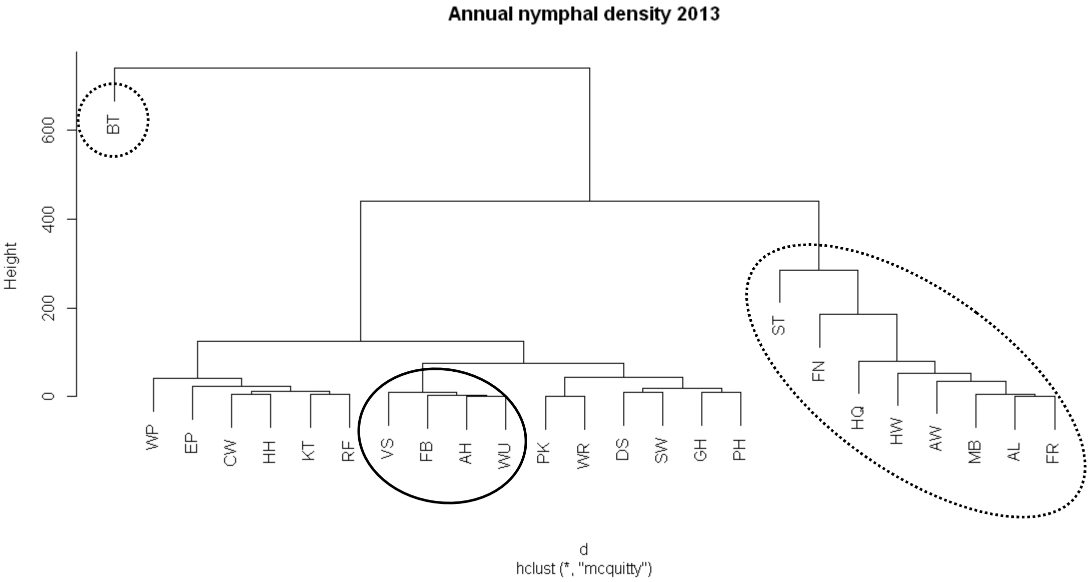
To verify the high variability of tick densities over the surveyed study sites, a **Kruskal-Wallis rank sum test** was performed based on the monthly tick data in each year. The results show highly significantly differences ($P < 0.001$) of nymphs (both years), adults (2013) and larvae (2014) as well as significantly differences ($P < 0.05$) of adults (2014) and larvae (2013).

Beside the observed variability in tick densities, also **groups of study sites with comparable annual nymphal densities** and **outliers** were identified using a cluster analysis. Figure 4.7 visualizes the results.

The group of very high annual nymphal densities (ovals with dotted lines) contains almost the same study sites in both years (SW is added in 2014) and most of them are located in or near the *Oberrheintiefland* (AL, AW, HQ, HW, FR, MB, ST). The sites are characterized by altitudes from 111 to 425 m a.s.l. (2013) and 111 to 610 m a.s.l. (2014), broad-leaved or mixed forest stands with a low to high herb and litter cover, mean annual air temperatures from 9.1 to 10.2 °C / 9.9 to 11.8°C (2013/2014) and mean annual relative humidity values from 85 to 93 % / 81 to 91 % (2013/2014).

Interestingly, only 28 % (7) of the 25 study sites are "moist habitats" (riparian forests or located in a depressed area, with a high groundwater level), but 71 % (5) of them are clustered within this group of very high tick densities (ST, FN, HQ, AW, AL) and, in addition, they represent 63 % of this group. In 2013, Botnang (BT) had a large discrepancy concerning all other sites based on its extremely high nymphal densities.

The other group (ovals with solid lines) is characterized by very low nymphal densities from 0 – 10 / 0 – 8 (2013/2014) nymphs. The composition of the group is more dynamic within the years except for the highest point *Feldberg (FB) and Allerheiligen* (AH), a coniferous dominated stand within large continuous forest areas of the *Schwarzwald*. It was surprising that a broad-leaved stand with moderate conditions (KT, mean annual T_A: 10 °C and RH: 92%) showed very low nymphal densities in 2014. All other study sites are characterized by intermediate tick densities.



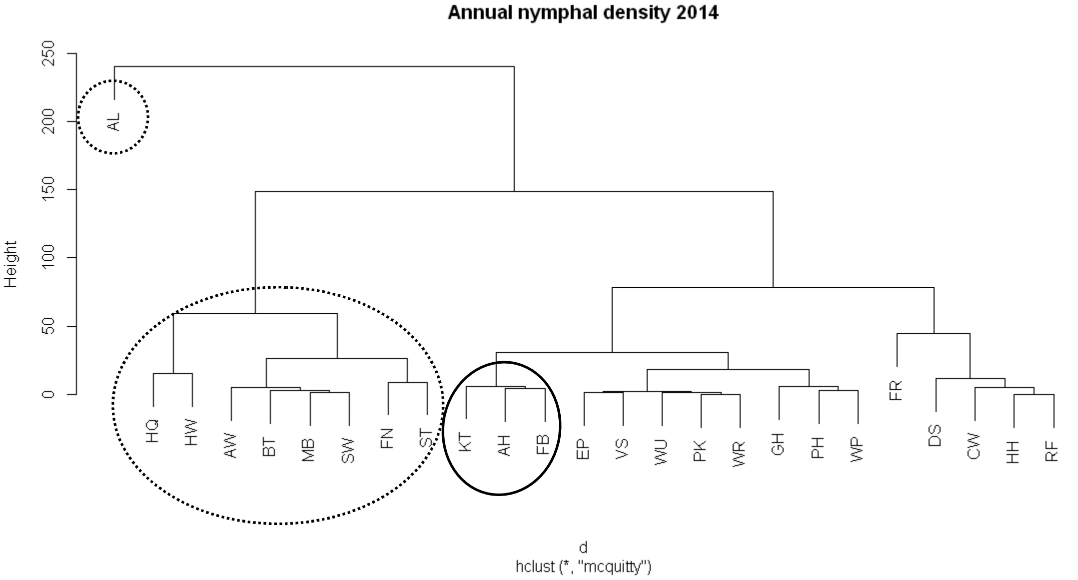


Figure 4.7: Results of the hierarchical cluster analysis with annual nymphal densities (corrected), illustrated by cluster dendrograms in 2013 (top) and 2014 (bottom). Ovals depict groups of very high (dotted) and very low (solid lines) densities. Note that study site "Neckaraue" (NA) is abbreviated with "HH" for processing reasons.

4.1.3 DISCUSSION AND CONCLUSIONS

Sections containing conclusions are highlighted with cursive characters.

SPATIAL PATTERN

Baden-Württemberg is located in the middle of the distribution area of *Ixodes ricinus*. Its different landscapes provide annual average temperatures comparable to conditions found at the ticks' Southern and Northern distribution boundaries, but most areas provide conditions in between.

One of the southernmost areas where *I. ricinus* has been found is the Rioja region in Spain (Estrada-Peña et al. 2004) with a long-term mean annual temperature of 13.9°C (data from weather station *Logrono Aeropuerto*, www.aemet.es, access on 23.10.2015). The warmest region in Baden-Württemberg is the *Oberrheintiefland*. As an example, the city of Karlsruhe has a long-term mean annual temperature of 11 °C (1981-2010; data from: www.dwd.de, access on 23.10.2015). The averaged precipitation is much lower in Logrono (405 mm/year) compared to Karlsruhe with (783 mm/year) and tick densities were high at almost all study sites located in and near the *Oberrheintiefland*. In conclusion, conditions should be far from those at southern distribution boundaries.

One of the northernmost study districts where the tick species have been found is *Stensele* in Sweden (Lindgren et al. 2000) with a long-term (1961-1990) mean annual temperature of 0.5 °C, located at 432 m a.s.l. (data from weather station *Arjeplog*: www.weather.gov.hk, access on 23.10.2015). The highest study site Feldberg is located at 1288 m a.s.l. and has a long-term (1981-2010) mean annual temperature of 3.9°C (data from: www.dwd.de, access on 23.10.2015), but despite its 3 K warmer conditions, no ticks were ever found.

The absence of ticks could be due to another factor: (Jaenson & Lindgren 2011) found that a vegetation period of 170 days on average along with a starting time in early May corresponds with the distribution boundary of *I. ricinus* in Sweden. At Feldberg, the duration of the vegetation period was lower in average (158 days) and started later (2013-06-04 and 2014-05-17). Besides, long periods of snow could promote the absence of ticks due to harder conditions for potential hosts. Since the duration of the vegetation period already reached the threshold of 170 days in 2014 at the highest study site Feldberg, I would expect this region to be settled by ticks over the next decades.

Altitude is an easily accessible but difficult to compare indicator for tick distribution boundaries. For example, at the Apennine Mountains in Italy the highest *I. ricinus* were found at 1650 m a.s.l. in 2009 to 2012 (Martello et al. 2014). In contrast, ticks occurred only up to 1100 m a.s.l. at the Krkonose Mountain in Czech Republic in 2002 to 2006 (Materna et al. 2008), which is more comparable to the tick situation in Baden-Württemberg. There are two relevant aspects that have to be considered:

(1) Due to global warming, a northern expansion (Lindgren et al. 2000, Jaenson & Lindgren 2011) and the occurrence of *Ixodes ricinus* ticks at higher altitudes (Daniel et al. 2003, Martello et al. 2014) combined with an increase of tick abundances have been documented over the last decades. Thus, older results may be no longer representative and this could partly explain the great altitude difference between results of Martello et al. (2014) and Materna et al. (2008).

(2) However, even more important seems to be the different geographic situation of the study sites. The Apennine Mountains are located in the warm Mediterranean part of Europe while the Krkonose Mountains are located far north with a latitude comparable to Cologne, Germany. It seems that going North and following reduced temperature conditions, the altitude limit of the tick distribution successively declines. Concluding, to compare distribution boundaries of *I. ricinus* Europe-wide, it seems necessary to use representative but more complex factors like the duration of vegetation period (Jaenson & Lindgren 2011) while comparisons with regions of similar macroclimatic conditions can be made by the factor altitude, given that the study is temporally comparable.

Temperature characteristics should be easier to compare but, if the resolution of the data differs, they first have to be adapted for further comparisons. For example, the report from Italy only gives minimum (January) and maximum (July and August) monthly mean temperatures of a official weather station near the ticks' distribution boundary. To compare conditions, these kind of temperature data were extracted from the official weather station near Feldberg. Results differed remarkably with a minimum of 1,9°C / -2,5°C in January and a maximum of 17,7°C / 11,5°C in July (Logrono / Feldberg 1996 – 2010, data from: www.dwd.de). Mean annual temperatures were given in the report by Materna et al (2008). A comparison of the conditions found at the stations that are close to the ticks' distribution boundary matched pretty well. The Czech station is characterized by a mean annual temperature of 1.9°C at 1362 m.a.sl., Feldberg by 3.9°C at 1288 m a.s.l. (1981-2010; data from: www.dwd.de).

The latter findings enforce that altitude dependent tick distribution boundaries and abundances have to be carefully surveyed and with respect to the climatic conditions of the area of investigation. Compared to the factor altitude, the long-term mean annual temperature is a more reliable estimator for the tick distribution boundary. A more precise predictor was the duration of the vegetation period, but calculations are time-consuming. For the future, long-term mean annual temperature values could instead be used as a standardized habitat characteristic. Doing this, it would be a lot easier to compare results from different regions and follow the expansion of I. ricinus in Europe due to global warming effects.

TICK PHENOLOGY

A bimodal activity pattern with a main peak in spring and a minor peak in autumn has frequently been described of *Ixodes ricinus* located in Central Europe and parts of Great Britain and a monomodal pattern for Ireland and South England (Belozerov 1982, Gray 1991, Kurtenbach et al. 2006). These statements contain the assumption of a region dependent activity pattern. In contrast, results for Baden-Württemberg show a high variance of activity patterns over the set of study sites and, in addition, over the years. Since the duration of this study is only 2 years, long-term observation could help to integrate the findings. Distinct inter-annual variances in nymphal activity were also found over a six year campaign from 1995 – 2000 at one study site in South England (Randolph et al. 2002). At the same time, a study was carried out at a site in the Rioja region in north-central Spain, showing a constant pattern of nymphal activity (Estrada-Peña et al. 2004). The findings in Baden-Württemberg correspond with the findings in south England. This leads to the assumption that the variability of activity patterns is in fact a regional phenomenon and probably linked to the long-term suitability of ticks' living conditions. The Spanish tick population is located near the species' southern distribution boundary and survival conditions are harsher due to drought. This very likely leads to a more specified behavior adaption, especially in summer where drought leads to a more constant and predictable water stress for ticks, and thus to stable activity pattern over the years. In contrast, survival and development conditions are more variable from year to year in the center of tick distribution, e.g. Central Europe, which seems to cause a higher behavioral variability and thus lead to diverse activity patterns. These findings are supported by results by Schulz et al (2014) who found diverse activity patterns of *I. ricinus* in 2011 in Baden-Württemberg and bordering German states. Furthermore, the delayed onset of tick activity in 2013 due to a cold spring coincides

with findings from (Perret et al. 2000) in Switzerland. There is one restriction that has to be mentioned, if the activity of ticks is described with tick data based on monthly flagging. An important demand on the flagging method is simultaneity (Milne 1943). Unfortunately, the tick data used in this thesis lack the demand of simultaneity to a great extent. The time of monthly flagging varies from days to weeks and, in addition, within the daytime over all 25 study sites. Resulting consequences for the interpretation of activity patterns, i.e. tick phenology, are currently unknown and most likely very heterogeneous (Estrada-Peña & de la Fuente 2014).
This is a major reason why no further analyses of activity pattern are carried out in this thesis. To obtain preferably comparable data for activity investigations in the future, it seems essential to combine flagging data with data from tick activity patches (method described in Dautel et al. 2008) and local microclimatic measurements to get an estimate about the proportion of flagged ticks and, in addition, to detect tick activity behavior that is related to specific weather events.

TICK DENSITY

Nymphal densities are not uniformly distributed over Baden-Württemberg but seem to cluster in different ecological landscapes. Constant high nymphal densities were found at most study sites in the lowlands *Oberrheintiefland* and bordering zones, constant low nymphal densities at the medium mountains *Schwarzwald* and *Schwäbische Alb*. Low densities on the medium mountains can be explained by a decrease of tick abundances with increasing altitudes according to unsuitable development conditions (Jouda et al. 2004, Materna et al. 2008). Constant high densities can be explained as follows: Study sites of the lowlands, warmest landscape of all, are mostly riparian forests or geomorphologically moist habitats. The combination of suitable temperature and humidity conditions seem to be relevant for the constantly high tick densities. In addition, some study sites were chosen to test the hypothesis that "moist habitats", such as riparian forests or forests located in a depressed area with a high groundwater level, provide very suitable conditions for ticks and thus lead to high tick densities (see also method chapter 3.1.). The cluster analysis provided results that support this hypothesis, since 85% (exclusive of the swamp) of the moist habitats were clustered in the group of highest nymphal densities. These are located in or near the *Oberrheintiefland* or near the lake *Bodensee*. These results are evidence for the high suitability of forest habitats providing warm and moist conditions and coincide with common knowledge about physiological needs of *I. ricinus* (see Chapter 2.1.4).

It was striking that the study sites showed significantly different tick densities in 2013 and 2014. Nymphs tended to decrease in numbers from 2013 to 2014. Numbers of larvae and adults were also significantly different in both years, but showed no clear trend of increase or decrease over all study sites. One explanation for these finding is: Nymphs develop from larvae of the previous year. Thus, many larvae must have developed successfully from 2012 to 2013, leading to high nymphal densities in 2013. Relating to findings from Ostfeld et al. (1996) and Ostfeld et al. (2006) for *Ixodes scapularis* nymphs in the US, a high fruit production of trees in 2011 could have been responsible for a high abundance of small mammal hosts in 2012 and thus high numbers of larvae successfully finding a suitable host. Subsequently, numbers of nymphs increase one year later – in 2013. Indeed, 2011 was a year with an intensive fruiting of forest trees, especially beech trees (Meining et al. 2011), which are common at most study sites. Therefore, the observed decrease of nymphal densities from 2013 to 2014 could be just a return to normal densities in 2014. In addition, increasing densities of adults from 2013 to 2014 (observed at 15 study sites) could be a direct result of the high nymphal densities in 2013. A possible negative influence of cold temperatures in winter 2013/2014 on tick survival is examined later in Chapter 3.4.2.

The observed indifferent density patterns of larvae can be explained by the high uncertainty of data gathered with the flagging method (Tack et al. 2011). Larvae are often clustered in nature and tend to quest near the ground surface so that the amount of collected larvae is more or less a result of chance. The decrease of nymphal densities was remarkable at study sites of lower altitude (< 600 m a.s.l.: 94%), while a large portion of sites located at high altitude showed an increase from 2013 to 2014. The winter 2013/2014 was extremely mild. Comparable observations were made by Lauterbach et al. (2013). They found that nymphal densities increased significantly at *Schwäbische Alb* on altitudes of 500 to 900 m a.s.l. after a winter with mild conditions (year 2008) compared to two other winters of much colder conditions with a difference of about 3 Kelvin in average (years 2009 and 2010). Results of this study also detected an increase of nymphal densities on altitudes of 500 m a.s.l. and higher after the very mild winter 2013/2014.A higher mortality of eggs in 2012 to 2013 and/or a higher mortality of larvae in 2013 could also have caused the observed decrease in nymphal densities 2014. Weather conditions that potentially caused high losses in 2013 were a dry spring (March and April) for developing eggs and resting larvae as well as a warm and dry summer for questing larvae. Since eggs and larvae are the development stages most sensitive to dehydration (Randolph 2004), dry conditions in summer likely caused the losses.

These considerations demonstrate well how complex the matter is and how much effort it costs to identify responsible factors affecting tick densities. High host abundances can increase the tick population, but effects are only detectable in the following years. Weather conditions can cause a high mortality, which is not directly measurable by flagging (since dead ticks are invisible ticks). Moreover, if weather occurrences affect the ticks' mortality at all is mainly a matter of the buffering potential a specific forest habitat has (see Chapter 2.2.) and therefore highly variable over the 25 study sites.

METHODS AND FURTHER APPROACH

The resulting number of ticks sampled with the flagging procedure depends on several influencing factors. For example, the effectivity of catching single tick stages in forest habitats is strongly connected to the height of flagging (Mejlon & Jaenson 1997, Boehnke 2011). Based on this knowledge, I invented a standardized flagging procedure to minimize negative effects of habitat characteristics in the field on the comparability of the resulting tick data. This procedure is restricted to forest habitats and has to be adapted first for an usage in other habitat types.

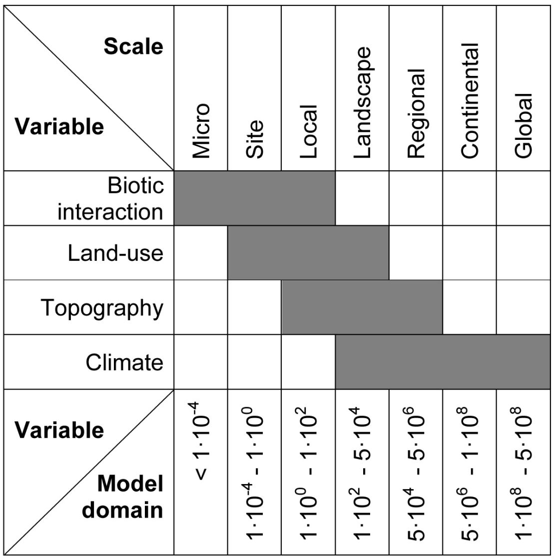
However, the amount of sampled ticks depends also on several other factors, such as season, weather conditions and the sampling method itself (Dantas-Torres et al. 2013). Therefore, an important demand on the flagging method is simultaneity (Milne 1943) which is not provided for the used tick data in this thesis. However, it is impossible to estimate resulting differences of daily tick occurrences at present (Estrada-Pena & De La Fuente 2014). In addition, it was found that flagging at daytime likely leads to a considerable underestimation of tick densities compared to flagging at night, since relative humidity conditions are in general better at night and most hosts are nocturnal leading to a higher proportion of active ticks at night (Randolph & Storey 1999). However, since almost all tick sampling field studies were carried out at day, resulting data are more comparable even if tick densities itself are underestimated.

The resulting tick densities per year have to be analyzed with respect to these impacts which hamper the comparability of the data. Especially moderate tick densities (not very low or very high) could potentially originate from similar population abundances. As a consequence, analyses focus on the impact of environmental factors on tick densities on study sites with remarkably low or high tick densities and on process oriented examinations (see Chapter 4.5).

4.2 ESTIMATING *IXODES RICINUS* DENSITIES ON THE LANDSCAPE SCALE

The following chapter presents contents of the author's publication in 2015 (Boehnke et al. 2015). It is a brief overview about the content of the study, including an introduction part, material and methods, results and discussion.

The study describes the estimation of the spatial distribution of questing nymphal tick densities of *Ixodes ricinus* over the study area of Baden-Württemberg, based on a generalized linear model (GLM) approach. The outcome is a high resolution map of questing tick densities in the years 2013 and 2014, which is a first important step to quantify the risk of tick-borne diseases in the future. Previous *I. ricinus* maps were based on quantitative as well as semi-quantitative categorisations of the tick density observed at study sites with different vegetation types or indices, all compiled on local scales or maps based on presence-absence data on a regional or continental scale. In contrast, this study provides a quantitative approach on the landscape scale.



Scale / **Variable**	Micro	Site	Local	Landscape	Regional	Continental	Global
Biotic interaction	▨	▨	▨				
Land-use		▨	▨	▨			
Topography			▨	▨	▨		
Climate				▨	▨	▨	▨
Variable / **Model domain**	$< 1 \cdot 10^{-4}$	$1 \cdot 10^{-4} - 1 \cdot 10^{0}$	$1 \cdot 10^{0} - 1 \cdot 10^{2}$	$1 \cdot 10^{2} - 5 \cdot 10^{4}$	$5 \cdot 10^{4} - 5 \cdot 10^{6}$	$5 \cdot 10^{6} - 1 \cdot 10^{8}$	$1 \cdot 10^{8} - 5 \cdot 10^{8}$

Figure 4.8: Environmental variables affecting tick densities at different spatial scales. Scales are characterized by the model domain in km², adapted from Pearson & Dawson (2003).

Figure 4.8 provides an overview of several environmental factors and their effects on tick densities at different spatial scales. Data-basis is the nymphal tick density (corrected) of all 25 study sites in 2013 and 2014, as introduced in the last chapter.

These nymphal densities (N, nymphs/100m^2) were used to develop and calibrate one separate Poisson regression model for each year:

$$log_e(N) = \beta_0 + \beta_1 H + \beta_2 T + \beta_3 RH + \beta_4 SD + \sum_{i=5}^{8} \beta_i LC_i \,.$$

The environmental variables height above sea level (H), temperature (T, annual average), relative humidity (RH) and saturation deficit (SD, long-term averages) as well as land cover classification (LC) were used as explanatory variables. These were selected due to their biological relevance for *Ixodes ricinus* ticks, which strongly depend on sufficient temperature and moisture conditions and a suitable habitat type.

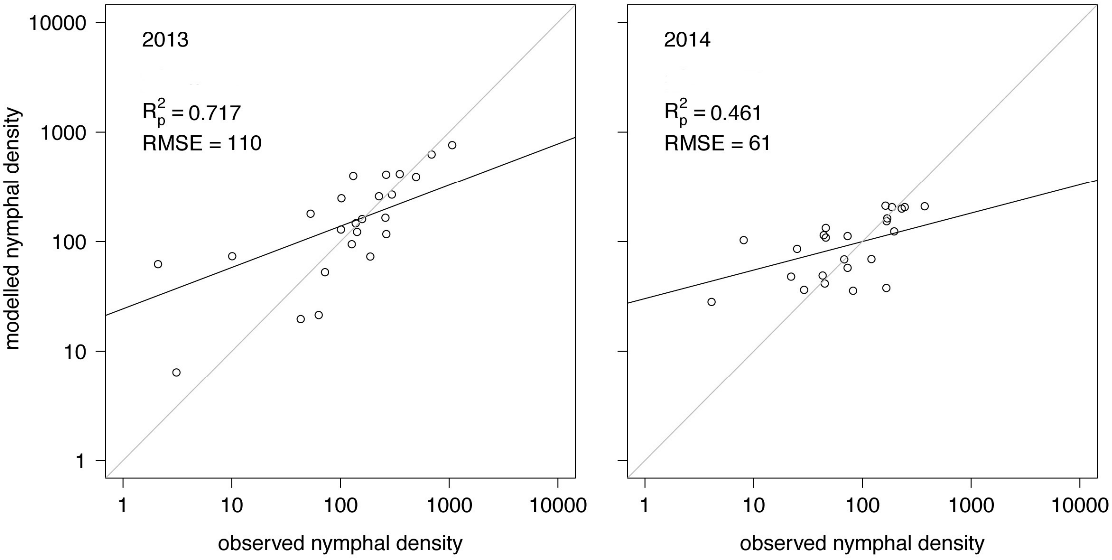


Figure 4.9: Comparison of observed vs. modelled nymphal densities for 2013 (left) and 2014 (right). The model performance is expressed by explained pseudo variances R_p^2 and root mean square errors (RMSE).

McFadden's pseudo Rp^2 (the coefficient of determination R^2, adapted for additional categorical variables such as LC) and the root mean square error (RMSE) were selected as goodness-of-fit measures. The final model was evaluated by leave-one-out cross-validation (LOOCV).

The model performance was expressed by explained variances of R_p^2 = 71.7% for the model fitted with the data of 2013 and R_p^2 = 46.1% for the model of 2014 (Figure 4.9). The results should be evaluated with regard to the uncertainties in observed tick densities as well as the low number of explanatory variables used for modelling. As a verification measure independent of observations used to calibrate the models, LOOCV errors of RMSE = 227 nymphs/100 m^2 for

2013 and RMSE = 104 nymphs/100 m^2 for 2014 were estimated. These errors are of the order of the mean nymphal density or 20 % of the maximum nymphal density, thus twice of the error estimated during the calibration process (Figure 4.9: RMSE = 110 nymphs/100 m^2 for 2013 and RMSE = 61 nymphs/100 m^2 for 2014). For high nymphal densities, these errors are comparable to the observational error, i.e. the accuracy of flagging.

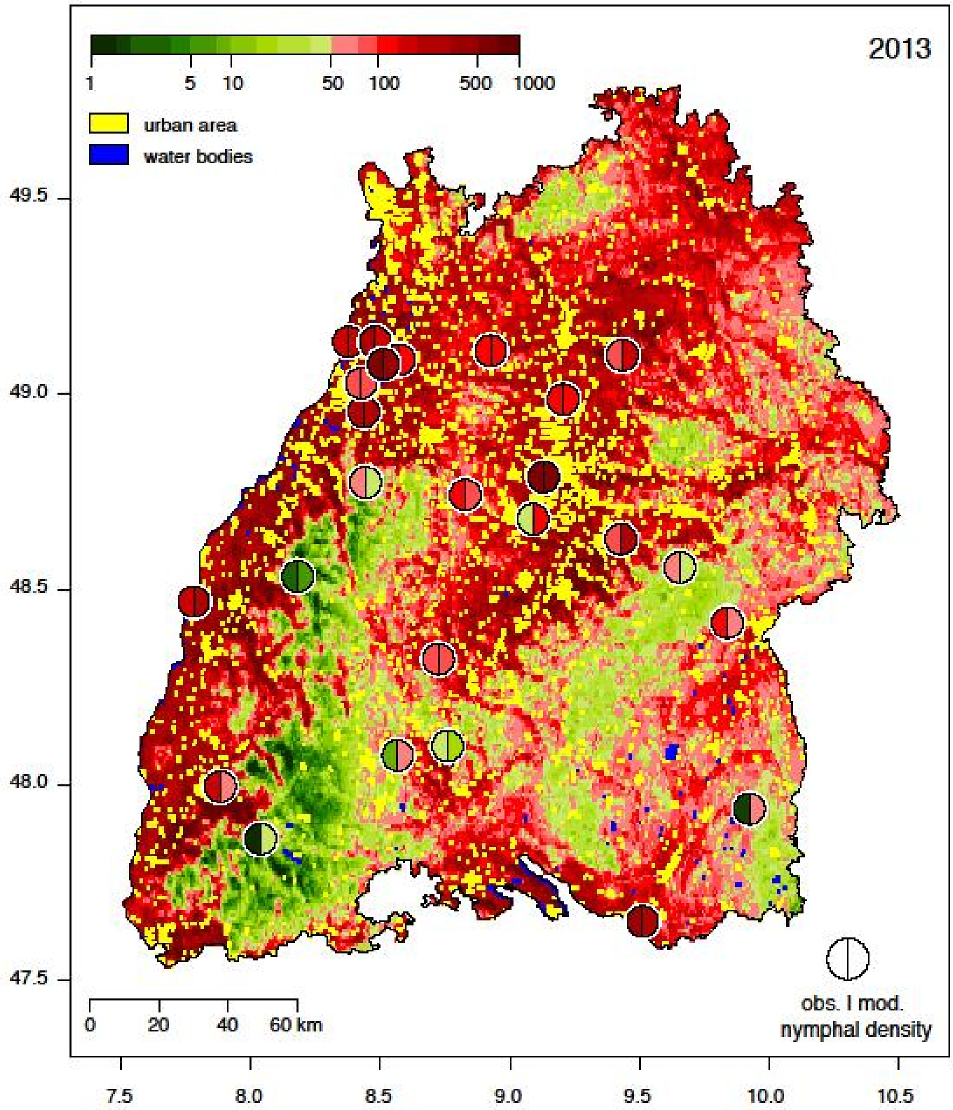


Figure 4.10: *Ixodes ricinus* nymphal ticks densities in 2013, interpolated to the entire region of Baden-Württemberg. Sampling locations are marked by a circle showing both the observed (left half) and the modelled (right half) tick density. The more similar the colors are, the better the models estimation power.

The resulting models were used to compile high-resolution maps with 0.5 km² grid size for the nymphal density distribution over Baden-Württemberg for 2013 (Figure 4.11).

Another map for the year 2014 is published in Boehnke at al. (2015). Each map depicts the spatial distribution of the density of nymphs and should be interpreted as the amount of *I. ricinus* nymphs that may be collected by monthly flagging an area of 100 m². Green grids represent locations with low nymphal densities (N = 0-50 nymphs/100 m^2), while red gradations indicate locations with high nymphal densities (N = 50-1000 nymphs/100 m^2). Low densities of *I. ricinus* are restricted to higher altitudes in the *Schwarzwald* characterised by slightly fragmented coniferous forest habitats.

Moderate densities were estimated for all other hilly countryside with altitudes around 300-800 m a.s.l. characterised by shorter growing periods and/or forested areas with high proportions of coniferous forest. Very high densities were estimated for the warmest parts at altitudes below 400 meters. This includes, in particular, the regions along the rivers *Rhine* and *Neckar* with surrounding areas, as well as the ambience of *Lake Constance*. The estimates for all other parts with warm to moderate climatic conditions lean towards high nymphal densities. Urban areas and water bodies were excluded from the analysis (Figure 4, yellow and blue areas). The comparison of the maps for 2013 and 2014 shows a similar distribution of the nymphal densities but generally lower nymphal densities in 2014, since the observed numbers of nymphs were significantly lower in 2014 compared to 2013 (compare Figure 4.5).

Uncertainties appeared in the modelling process, especially in the selection of explanatory variables. As suggested by Estrada-Pena et al. (2015), only biologically meaningful variables were considered instead of the frequently applied approach of reducing significant variables from a large set of possible explanatory variables. As a side effect, factors are not independent. Collinearities (e.g. between altitude and temperature) were estimated, which should be avoided in generally applicable models (Estrada-Pena et al. 2015).

Due to the low number of explanatory variables used in the model, this is, however, not possible without reducing the model's performance. Thus, the biological significance was valuated more highly than statistical features and no generally applicable regression parameters were given. Instead, the regression parameters were separately modeled for each year and must be modeled again if further years will be investigated. In doing so, the model is evidently usable for mapping, i.e. spatial interpolation, but could not be used to predict future scenarios. However, this is the first map that illustrates the geographical density distribution of *I. ricinus* for Baden-Württemberg, rather than the well-known map of TBE-risk areas (Figure 2.14) and the first *I. ricinus* tick density map with such a high spatial resolution.

4.3 Temperature and tick densities

This chapter focuses only on the microclimatic temperature factor, since this factor is known for its prominent influence on ticks living processes, their distribution and abundance (Dautel 2010).

The previous chapters examine the impacts of temperature on seasonal tick activity as well as the relationship between tick density and altitude. A clear decrease of tick density with increasing altitude was observed, in accordance with previous findings.

In the following chapter, the influence of the factor temperature on tick densities is examined through various conventional and enhanced methods:

First, a general impact of temperature-related parameters on tick densities is examined by annual values and altitude. Two altitude-dependent zones that offer different living conditions for ticks are detected by several statistical methods, along with challenges of this differentiation for further analysis (chapter 4.3.1).

Next (4.3.2), possible influences of winter temperatures on tick densities are analyzed. Since larvae and nymphs were strongly reduced in numbers from 2013 to 2014, possible mortality factors were tested for the relevant wintertime from October to April.

An analysis of the temperature conditions within the course of one tick season is performed in the last chapter 4.3.3. Temperature-related development rates are used to compare temperature conditions for all study sites. It is, to the knowledge of the author, the first time that development rates were used for this matter.

4.3.1 NYMPHS, ALTITUDE AND ANNUAL AVERAGE TEMPERATURE

Tick densities decreased considerably with growing altitude and falling mean annual air temperature in 2013 and 2014 (Figure 4.11).

To verify these visual observations, Spearman's rank correlation coefficients were calculated and a simple linear regression was carried out. Main results are presented in Table 4.3.

Table 4.3: Linear regression model quality (adjusted R-squared) and significance (P-value) as well as Spearman's rho (r_s, grey cells) for nymphal densities (ND) in 2013 and 2014 in relation to mean annual air / soil temperature and altitude.

	ND 2013		**ND 2014**		**ND 2013**	**ND 2014**
	adj. R^2	P-value	adj. R^2	P-value	r_s	r_s
air temperature	0.347	0.0015 **	0.364	0.0011 **	0.784 ***	0.637 ***
soil temperature	0.321	0.0023 **	0.322	0.0022**	0.734 ***	0.642 ***
altitude	0.335	0.0018 **	0.306	0.0030 **	-0.747 ***	-0.600 **

Significances: ** P < 0.01; *** P < 0.001

Spearman`s test results (r_s) indicate a highly significant negative correlation between the observed annual tick densities and altitude as well as a highly positive correlation with mean annual temperature values. Each linear regression model in itself explained significantly over 30% of the variance (Table 4.3) of nymphal densities in both years. Since the mean annual temperature is naturally strongly altitude dependent, the linear regression models of 2013 and 2014 explained almost all of the variance of the air temperature on a high significant level (2013: 90.5%, P < 0.001; 2014: 89.0%, P < 0.001) by altitude (see Figure 4.11c).

However, the scatter plots (Figure 4.11a/b) rather indicate an exponential relationship than a linear one between nymphal densities and temperature/altitude. In 2013, the exponential models resulted in a higher explained variance (air temperature = adj. R-squared: 0.420, P < 0.001***; altitude: = adj. R-squared: 0.404, P < 0.001***) compared to the linear models. In contrast, the exponential models resulted in a minor explained variance for 2014 (air temperature = adj. R-squared: 0.263, P < 0.01**; altitude: = adj. R-squared: 0.215, P < 0.05*). Since the data showed no consistent improvement using the exponential model, another approach was processed.

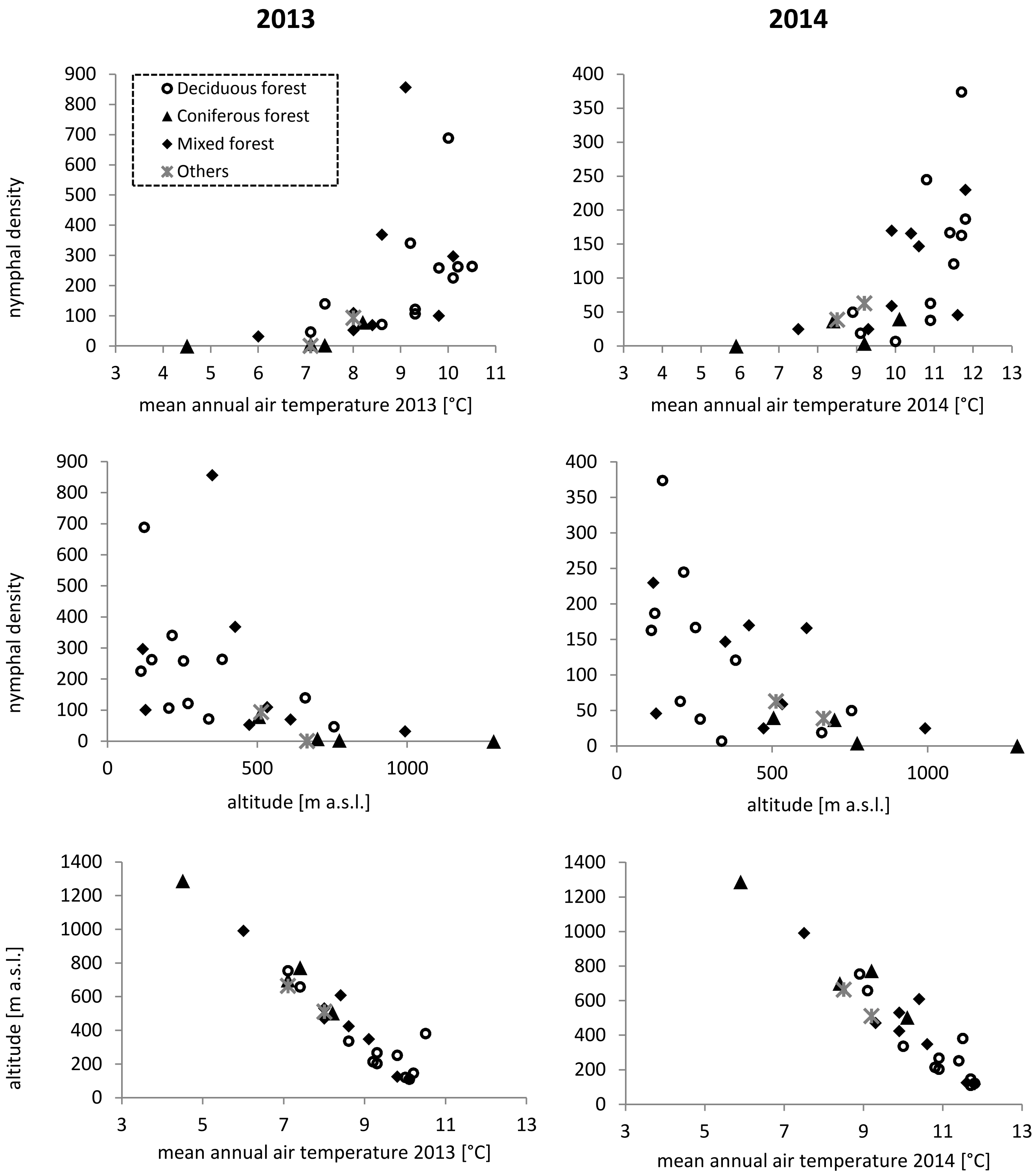


Figure 4.11: Relation between the annual nymphal density and 1. the mean annual temperature, 2. altitude of the study sites and 3. altitude and temperature separated after the years 2013 (left) and 2014 (right).

4.3.1.1 MARGINS OF TEMPERATURE DEPENDENT EFFECTS

According to the Mann-Whitney U Test, the population means between tick densities (separated after life stage) sampled at study sites lower than 500 m a.s.l. differed significantly (P < 0.05*) and high significantly for nymphs in 2013 (P < 0.001***) from those sampled at study sites higher than 500 m a.s.l.

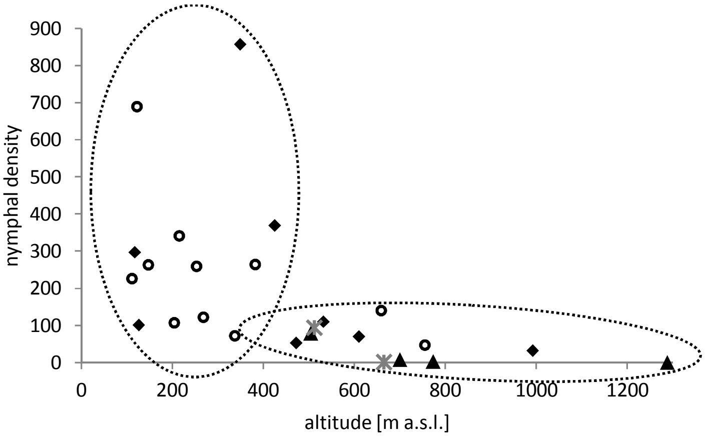


Figure 4.12: Extent of tick density variation (ovals) depending on altitude.

In addition, study sites below 500 m a.s.l or less (mean annual temperatures > 8.5 – 9°C) showed a high variability in nymphal densities in both years, while densities remained at a constant low level above 500 m a.s.l. (see Figure 4.12). Thus, linear regression models were used again with data subdivided into study sites higher (n = 11) and lower (n = 13, outlier removed) than 500 m a.s.l., using a piecewise linear regression. Table 4.4 illustrates the results.

Table 4.4: Output of the linear regression models and Spearman's rho when study sites are subdivided into groups of altitudes higher (High) and lower (Low) as 500 m a.s.l.. Non-significant results are shown on a grey background.

		ND 2013		ND 2014		ND 2013	ND 2014
		adj. R^2	P-value	adj. R^2	P-value	r_s	r_s
HIGH	air temperature	0.199	0.0946 .	0.312	0.0433 *	0.668 *	0.596 .
	soil temperature	0.135	0.1438	0.275	0.0564 .	0.396	0.770 **
	altitude	0.247	0.0687 .	0.138	0.1409	-0.736 *	-0.618 *
LOW	air temperature	-0.016	0.3877	0.081	0.1786	0.334	0.292
	soil temperature	-0.014	0.3817	0.008	0.3173	0.346	0.236
	altitude	-0.050	0.5273	0.012	0.3066	-0.197	-0.296

Significances: . P < 0.1; * P < 0.05

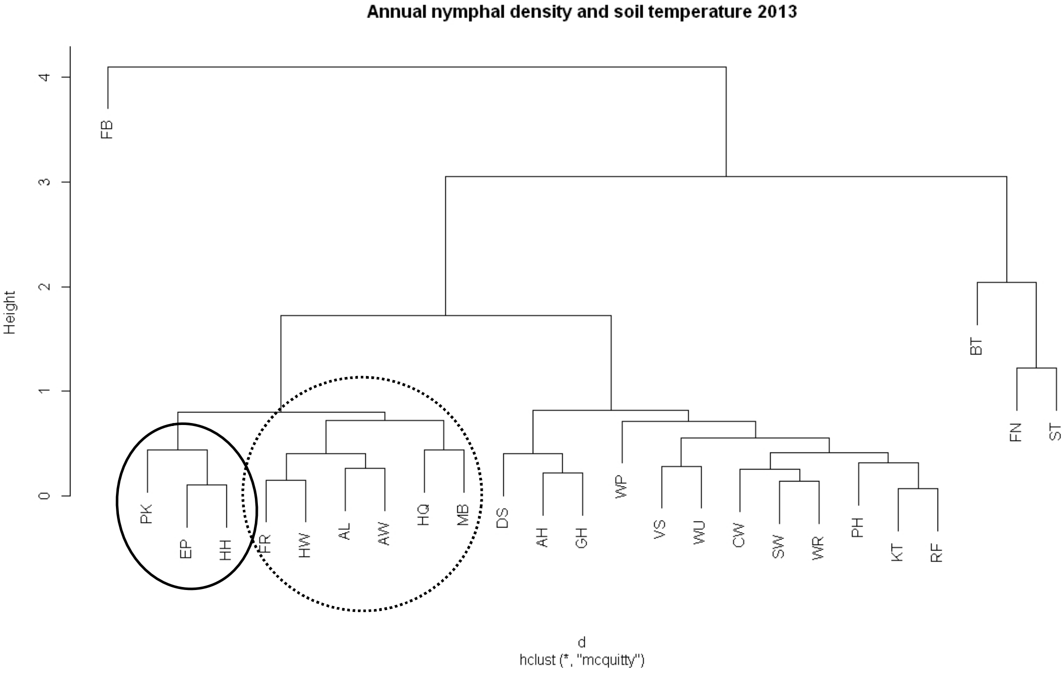
The linear models were also used to **calculate threshold values** when nymphal densities reach zero (the line crosses the x-axis). Threshold altitudes were calculated to be 990 m a.s.l. (full model) and 1100 m a.s.l. (high model) for the cooler year 2013. In the warmer year 2014, the threshold values increased to higher altitudes of 1160 and 1230 m a.s.l., respectively. The results widely agree with the field observations. Threshold values for mean annual temperature were 5.9 and 5 °C (full and high) for 2013 and 7 and 6.4°C (full and high) for 2014.

Using a **cluster analysis**, the high variability of nymphal densities observed at lower altitudes is analyzed. Results for nymphal densities and soil temperatures are depicted in Figure 4.13.

In both years, Feldberg (FB) was separated as the study site with the lowest temperature and no ticks, while the study sites with extraordinary high nymphal densities and warm conditions changed from Botnang (BT), Staffort (ST) and Friedrichshafen (FN) in 2013 to Altenheim (AL) in 2014.

The **first group** of study sites at lower altitudes (Figure 4.13, dotted lines) is characterized by warm conditions (2013/2014: 9.6-12.2°C) and high nymphal densities (226-351 nymphs in 2013 and 121-245 in 2014). The group contains all study sites of the *Oberrheintiefland* and its bordering transition zone (AW, HW, HQ, FR, MB, ST). The **second group** of study sites at low altitudes (Figure 4.13, solid lines) is also characterized by warm conditions (2013/2014: 9.7-11.7°C) but, in contrast to the first group, low nymphal densities (101-158 nymphs in 2013, 44-73 in 2014). The group is constantly built up by three study sites, containing 66% of the sites within the *Neckar-Tauber-Gäuplatten* (NA, EP) and a pine-beech stand in Karlsruhe (PK).

All **other groups** reflect cooler conditions compared to the latter or cooler conditions combinded with comparably low tick densities, e.g., the study sites Kirchheim Teck (KT) and Parzelle Hohenheim (PH) located at medium altitudes of 337 m and 472 m.a.s.l., respectively (2013/2014: 9.1-10.2°C and 8 to 131 nymphs).



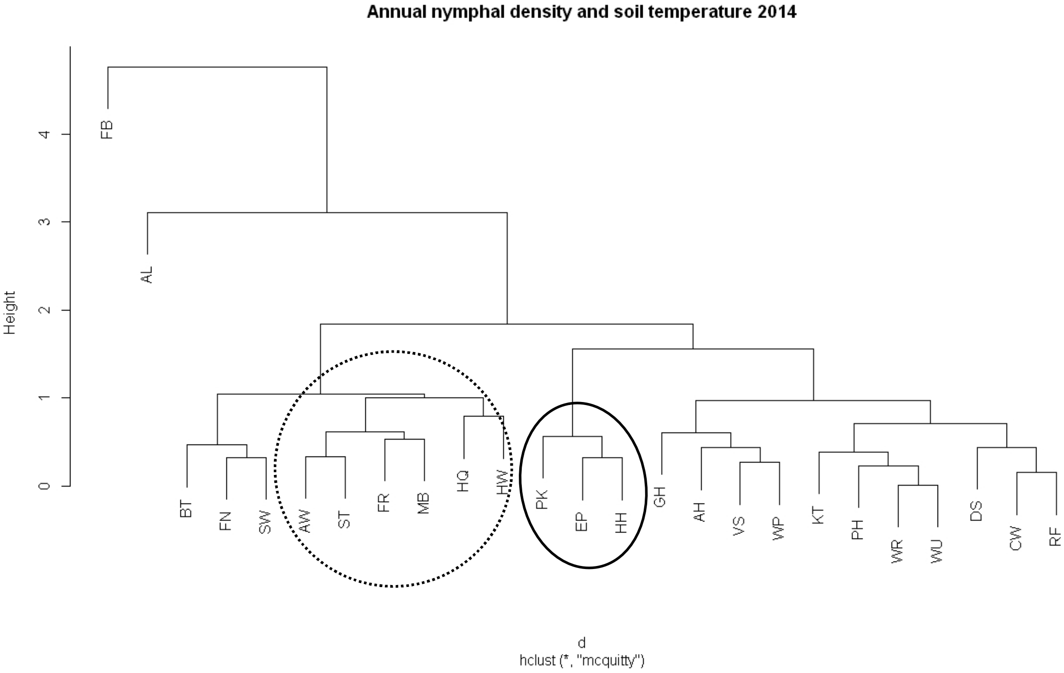


Figure 4.13: Results of the hierarchical cluster analysis based on annual nymphal densities (corrected) and mean annual soil temperatures, illustrated by cluster dendrograms in 2013 (top) and 2014 (bottom). Ovals depict groups of very high (dotted) and very low (solid lines) nymphal densities at study sites of low altitude. Note that study site "Neckaraue" (NA) is abbreviated with "HH" for processing reasons.

4.3.1.2 HOW TEMPERATURE MARGINS CONSTRAIN FURTHER ANALYSIS

In this context, also the **forest type** has to be considered as an important influence factor on tick densities.

The visual examination of Figure 4.11 indicates that coniferous forests had constantly low nymphal densities whereas mixed and broad-leaved forests showed a high variability in both years. The strength of association between the forest type and nymphal density was evaluated using a **multiple regression**. A significant model result was only detected for data in 2013 (Results in Table 4.5).

Table 4.5: Summary of multiple regression models for all study sites in 2013 and 2014. Nymphal density is the dependent variable. For each explanatory variable (forest type) the regression coefficient β, the standard error SE, the t-value (test statistics) and the p-value (significance) are given. Forest types are: B = broad-leaved, C = coniferous, M = mixed. Type A is set as default ($\beta=0$).

2013	β	**SE**	**t-value**	**p-value**	
(Intercept)	64.50	107.27	0.601	0.554	**p (model)**
Forest B	183.68	116.61	1.575	0.131	0.0936 *
Forest C	-35.75	131.38	-0.272	0.788	**adj. R2**
Forest M	107.50	121.63	0.884	0.387	0.16
2014					
(Intercept)	59.00	51.22	1.152	0.263	**p (model)**
Forest B	52.20	56.11	0.930	0.363	0.168
Forest C	-35.75	62.73	-0.570	0.575	**adj. R2**
Forest M	57.12	57.27	0.998	0.330	0.10

Significances: * $P < 0.05$

Coefficients (β) indicate that the parameter “coniferous forest” had a negative influence whereas “broad-leaved” and “mixed forest” had a positive influence on nymphal densities. However, only the model in total was significant, not the coefficients of singular parameters – that weaken the latter statements on the influence of different forest types.

Since former analysis detected a strong dependence for tick densities at study sites in areas of high altitude, the next step was to use **sub settings of the study sites** according to their altitude. Neither the multiple regression analysis for high study sites (> 500 m.a.sl.; $P_{2013} = 0.39$; $P_{2014} = 0.37$) nor for low study sites (< 500 m.a.sl.; $P_{2013} = 0.72$; $P_{2014} = 0.88$) showed significant associations between the forest type and nymphal densities.

To interpret these results correctly, the **spatial distribution of the factor forest type** has to be considered.

Among the 25 selected study sites, coniferous forests are restricted to altitudes above 500 m a.s.l., mixed forests occur in a range of 349 to 992 m a.s.l and broad-leaved forests are dominant at altitudes below 400 m a.s.l. (Table 4.1; Figure 4.11). Looking at the distribution of forest type, it is striking that all coniferous forests and other habitat types (swamp and clearing) are restricted to altitudes above 500 m a.s.l. (see Figure 4.14).

Concluding, the detected negative influence of coniferous forests on nymphal densities could just be an artifact of the temperature-dependent altitude effect that was pointed out just before.

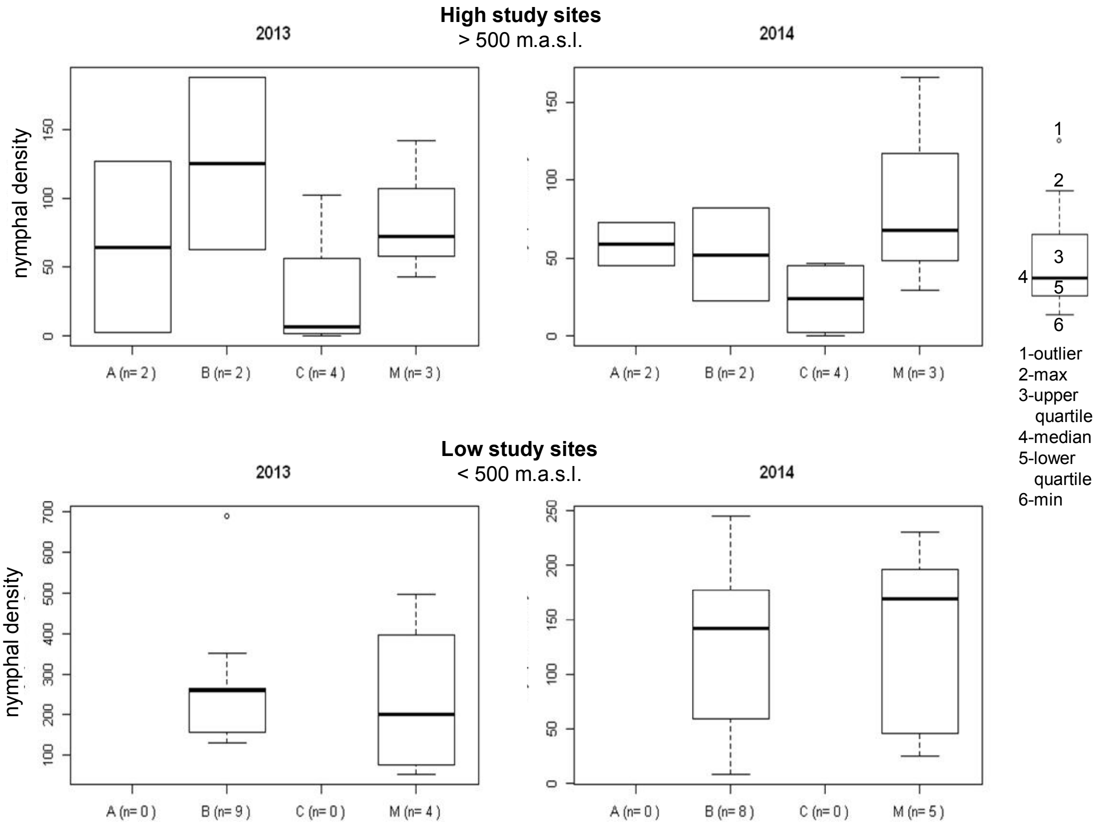


Figure 4.14: Distribution of nymphal densities according to forest type (A = others, B = broad-leaved, C = coniferous, M = mixed) and altitude of the study sites.

4.3.2 PREDICTION OF TICK MORTALITY IN WINTER

A strong decrease of questing *Ixodes ricinus* abundances was detected in the year 2014 compared to 2013. This reduction could be caused by several stress factors in winter:

a) freezing conditions
b) temperature variations
c) ice contact

The winter period of 2012/2013 was excluded from analysis because no complete tick data set exists for 2012.

4.3.2.1 MORTALITY CAUSED BY FREEZING CONDITIONS

Tick mortality risk increases through cold temperature conditions (<-10°C), especially if they are ongoing (Dautel & Knülle 1997). Thus, datasets were screened for values of less than or equal to -10°C. Table 4.6 illustrates the results.

Table 4.6: Absolute minimum air (T_A) and soil (T_S) temperatures based on 10 minute values measured from 2013-10-01 to 2014-03-31.

		Absolute minimum	
Site	**Altitude**	**T_A**	**T_S**
FB	1288	-8,0	0,6
GH	992	-8,5	1,1
AH	773	-0,8	2,3
DS	755	-9,3	1,6
VS	700	-7,7	2,6
WU	665	-11,5	2,7
WP	659	-8,0	2,5
SW	610	-6,2	3,4
CW	532	-6,1	2,5
RF	512	-9,1	0,6
WR	504	-5,2	0,7
PH	472	-6,5	2,2
FH	425	-5,9	2,3
FR	382	-4,7	4,1
BT	349	-5,2	2,6
KT	337	-6,8	1,0
EP	268	-5,6	3,4
MB	253	-2,8	3,3
HQ	215	-4,4	2,5
NA	204	-4,8	1,3
AL	147	-4,8	4,5
PK	126	-5,4	2,5
ST	122	-5,4	1,9
HW	117	-3,1	3,7
AW	111	-3,0	2,1

Long periods with temperatures below -10°C were not observed in winter 2013/2014. Air temperatures (50 cm height) below -10°C occurred temporarily at the swamp study site (WU). Close to -10°C were temporarily found at a more highly elevated broad-leaved (DS) and open mixed forest stand (GH) as well as at the clearing (RF). No data exist for AH from 20th October to 18th February and for FB from 24th November to 22nd January, thus lowest absolute minimum temperatures are probably not recorded. Other study sites never reached air temperatures below -8°C. Thus, periods of very low temperatures that would increase tick mortality were not observed.

4.3.2.2 MORTALITY CAUSED BY TEMPERATURE VARIATIONS

According to findings of (Herrmann & Gern 2013), frequent **temperature variations within a day** between -5° and +10°C damage ticks and lead to a more highly mortality. Thus, the air temperature data set was screened for those conditions. They occurred only at two sites: at the swamp (WU) on 3 days in December 2013 and on 9 days from February to April 2014 and at the clearing (RF) habitat on two days in total (one in February, one in April 2014). In addition, the litter temperatures measured at the Intensive Stations (not SW) never reached the lower value, with a minimum of only -2.4°C.

Winter temperatures that vary strongly within a day were rarely observed and never to a frequent, tick damaging extent. They occurred only under atmospheric conditions at study sites with sparse (the swamp, WU) or no (the clearance, RF) tree coverage. Concluding, this kind of tick damaging situation was very likely not responsible for the decrease of nymphal densities.

4.3.2.3 MORTALITY CAUSED BY ICE CONTACT

Direct ice contact can cause the “**inoculative freezing**” in times of frost occurrence (Dautel 2010). *Ixodes ricinus* larvae and nymphs died after 24 hours of ice exposition at -6°C (Dautel & Knülle 1997). The number of days with possible frost occurrences in the litter layer (T < 0°C) and freezing temperatures in 50 cm height are summarized in Table 4.7.

No litter frost was detected at the sites **HW** (117 m a.s.l.) and **MB** (253 m a.s.l.). However, three days of litter frost occurred in January and February 2014, respectively, at the site **AW** (111 m a.s.l) which is located only about 5 km away from HW. Thus, an air temperature below 0°C does not automatically lead to litter frost (< 0C° litter temperature), since such temperatures were often observed in HW and MB in the air but never in the litter layer.

Table 4.7: No. of days per month with a high chance of frost (temperature <0°C) in the litter layer (T_L) at study sites with an Intensive Station. In addition, no. of days with air temperatures below 0°C (T_A, in 50 cm) is given along with no. of days with existing data (n). The soil temperature never fell below 0°C and is therefore not listed. No data exist for the litter temperature in SW (NA).

	Oct			Nov			Dec			Jan			Feb			Mar			Apr		
	T_L	T_A	***n***	T_L	T_A	***n***	T_L	T_A	***n***	T_L	T_A	***n***	T_L	T_A	***n***	T_L	T_A	***n***	T_L	T_A	***n***
AW	0	0	*31*	0	1	*20*	0	12	*21*	3	11	*31*	3	10	*28*	0	6	*31*	0	0	*30*
HW	0	0	*31*	0	5	*30*	0	16	*31*	0	11	*31*	0	9	*28*	0	6	*31*	0	1	*30*
MB	0	0	*31*	0	5	*30*	0	12	*31*	0	7	*31*	0	5	*28*	0	2	*31*	0	0	*30*
SW	NA	0	*31*	NA	7	*30*	NA	11	*31*	NA	8	*31*	NA	1	*28*	NA	2	*31*	NA	0	*30*
DS	0	0	*0*	4	15	*24*	11	17	*31*	6	17	*31*	9	16	*28*	4	6	*31*	1	2	*30*

Unfortunately, no litter data exist for SW due to technical issues. However, SW (610 m a.s.l.) had fewer days with air temperatures below 0°C than all other study sites, so litter frost occurrences were unlikely a relevant problem for ticks.

At the study site DS (755 m a.s.l.), litter frost occurred from November to April on 35 days in total and the air temperature fell below the freezing temperature on 73 days. The litter layer in DS was temporarily shielded against falling temperatures by a snow cover.

4.3.2.4 PROCESSES BEHIND FROST OCCURRENCES

Screening the data for frost occurrences in the litter in DS led to an interesting finding: Soil frost was sometimes combined with air temperatures (50 cm) below 0°C, sometimes not (see Figure 4.15).

Hence, there exist different mechanisms that lead to a temperature drop down in the litter layer. If the litter temperature follows a decreasing air temperature and reaches 0°C and below, frost is caused by advection (left graphic). If the temperature remains more highly in the air than in the litter at night, frost emerges most likely from energy losses at the ground surface via nocturnal radiation (right graphic) (see Chapter 2.2.2). Of course, besides a low temperature a sufficient amount of water stored within the litter layer is needed to cause frost. Following considerations are made under this assumption.

In Drackenstein (DS), advection processes probably causing soil frost were observed mainly from December 2013 to early February 2014, with air temperatures down to -5°C as a minimum. Sometimes, soil frost kept on for days when the air temperature remained below 0°C.

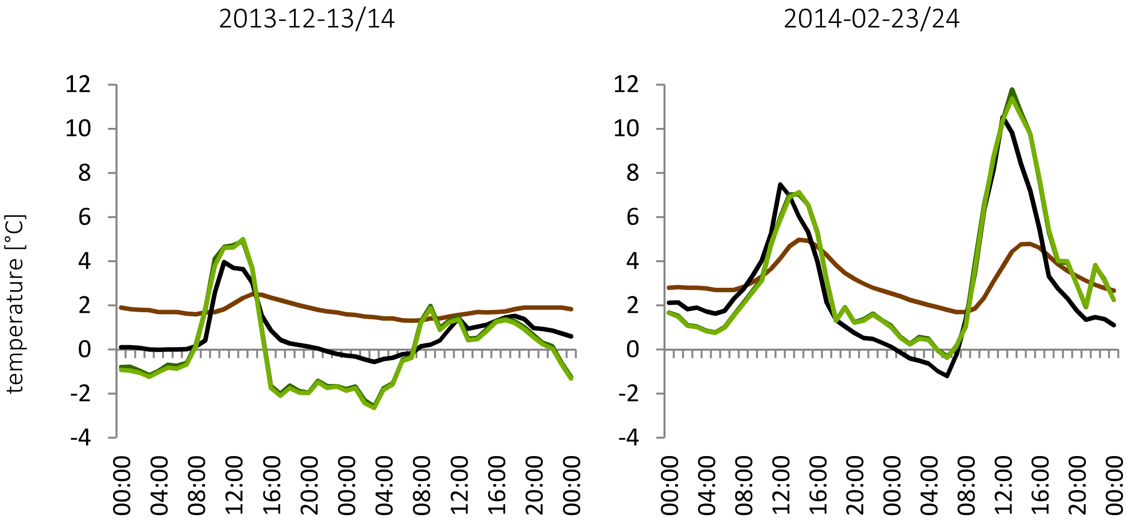


Figure 4.15: Temperature course in the leaf litter (black), in 50 cm height (green, light (1) and dark (2)) and in 5 cm soil depth (brown) during litter frost events (T < 0°C) caused by a) advection (left, the temperature in the litter follows the air temperature) and by b) nocturnal radiation (right, the air temperature remains warmer than the litter) in Drackenstein (DS).

Frost caused by **nocturnal radiation** was observed more frequently than the latter. It was identified if the litter temperature dropped down earlier and to a higher extent than the air temperature at night. These temporarily processes occurred mainly in February and March 2014 when the soil temperature was between 0° and 2.3 °C. Radiation processes cooled down the litter temperature to -1.7°C as a minimum.

Schwarzwald (**SW**), which is the study site that provides data at a comparable altitude to DS, unfortunately lacks litter data for this period. However, the air temperature was mostly higher than 0°C in SW, so **advection** processes unlikely caused frost. Frost caused by **nocturnal radiation** should also occur to a lesser extent, because the radiation is hindered due to foliage in the partly evergreen forest (SW) compared to the completely defoliated broad-leaved forest (DS).

Litter frost events caused by advection processes were observed in January and February at **AW** (111 m a.s.l.), when air temperatures decreased down to -2.8°C at minimum. Diurnal radiation processes were not observed. No frost events occurred at the nearby stations **HW** and **MB**.

4.3.3 PREDICTION OF TEMPERATURE DEPENDENT DEVELOPMENT RATES

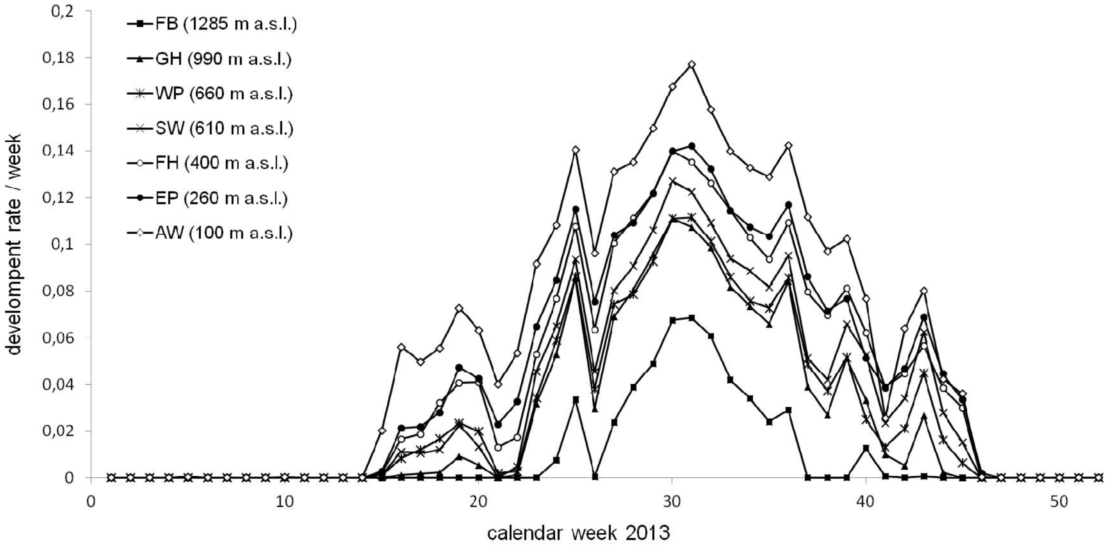


Figure 4.16: Seasonal course of Ixodes ricinus egg-to-larvae development rates per week trough 2013 at the two highest study sites (FB) and (GH), the lowest study site (AW) and at sites of intermediate altitude in broad-leaved (EP, WP) and mixed (FN, SW) forest stands. Calculations have been made with soil temperature in 5 cm depth.

Figure 4.16 illustrates the **egg development** of *I. ricinus* in the course of the year 2013. The **weekly development rate** is highest in summer, its course basically shows the annual temperature variations through the year 2013. Nearly 20% of egg development was completed within one week in summer at the warmest location Auwald (AW) and as far as 15% at study sites up to 400 m a.s.l. (EP and FN). At the beginning of the development phase, the development is comparably slow with 4 to 8 % per week due to the very cold conditions in spring 2013. A cold phase in May lead to a break of 2 weeks in development at study sites above 600 m.a.s.l.. Interestingly, development has a very similar extent at the study sites in 660 m a.s.l (WP) and 990 m a.s.l. (GH) but, in contrast, has a very differing course at the highest study site, Feldberg (FB).

Figure 4.17 lists several **daily development rate dependent indices** for each study site in the years 2013 and 2014. In 2013, **egg development started** more or less simultaneously within two calendar weeks (15 -16) at all study sites except for Feldberg (FB), where the start was delayed for about two month. In contrast, the starting date in 2014 varied from calendar week 10 at the warmest site to week 15 at Gosheim (GH) and, again, delayed for two month more at FB. Conspicuously, the time differences between the starting in 2013 compared to 2014 are smaller for study sites located in areas of high altitude compared to those at low altitude.

			AW	HW	ST	PK	AL	NA	HQ	MB	EP	KT	BT	FR	FN	PH	WR	RF	CW	SW	WP	WU	VS	DS	AH	GH	FB
	altitude [m a.s.l]		111	117	122	126	147	204	215	253	268	337	349	382	425	472	504	512	532	610	659	665	700	755	773	992	1288
2013	Developm starts	KW	15	15	15	-	15	15	-	15	15	-	-	-	15	-	16	15	-	16	16	-	16	16	-	16	24
		Month	Apr	Apr	Apr	-	Apr	Apr	-	Apr	Apr	-	-	-	Apr	-	Apr	Apr	-	Apr	Apr	-	Apr	Apr	-	Apr	Jun
	DrR reaches 50%	KW	24	25	24	-	24	25	26	25	26	-	-	-	27	-	29	25	-	29	29	-	29	29	-	30	37
		Month	Jun	Jun	Jun	-	Jun	Juni	Jun	Juni	Jun	-	-	-	Jul	-	Jul	Juni	-	Jul	Jul	-	Jul	Jul	-	Jul	Sept
	DrR reaches 1	KW	28	29	28	-	28	30	31	30	30	-	-	-	31	-	34	29	-	33	34	-	34	34	-	36	-
		Month	Jul	Jul	Jul	-	Jul	Jul	Aug	Jul	Jul	-	-	-	Aug	-	Aug	Jul	-	Aug	Aug	-	Aug	Aug	-	Sept	-
	Developm ends	KW	-	47	46	46	47	46	45	46	46	-	-	-	46	45	45	45	-	45	45	45	45	-	-	44	-
		Month	-	Nov	Nov	Nov	Nov	Nov	Nov	Nov	Nov	-	-	-	Nov	Nov	Nov	Nov	-	Nov	Nov	Nov	Nov	-	-	Nov	-
	DdR at 31.12.2013		1,8	**2,6**	**2,7**	1,1	**2,8**	**2,4**	2,0	**2,4**	**2,3**	1,5	2,0	2,3	**2,1**	1,2	**1,6**	**2,2**	1,1	**1,7**	**1,5**	1,5	**1,5**	1,2	**-**	**1,3**	0,5
	Nymphs / 100m²		226	297	689	101	263	138	351	259	158	131	1066	264	496	53	102	127	142	72	188	2	10	63	3	43	0
2014	Developm starts	KW	10	11	11	12	11	12	12	11	12	12	12	11	14	14	14	12	14	12	14	14	15	13	14	15	23
		Month	Mrz	Mrz	Mrz	Mrz	Mrz	Mrz	Mrz	Mrz	Mrz	Mrz	Mrz	Mrz	Apr	Apr	Apr	Mrz	Apr	Mrz	Apr	Apr	Apr	Mrz	Apr	Apr	Jun
	DrR reaches 50%	KW	21	23	21	23	22	23	24	23	24	24	25	23	25	27	28	24	28	26	28	24	29	27	29	29	36
		Month	Mai	Jun	Mai	Jun	Jun	Jun	Jun	Jun	Jun	Jun	Jun	Jun	Jun	Jul	Jul	Jun	Jul	Juni	Jul	Jun	Jul	Jul	May	Jul	Sept
	DrR reaches 1	KW	25	-	26	28	26	28	30	28	29	29	30	28	30	32	33	29	33	32	33	29	34	32	35	36	-
		Month	Jun	-	Jun	Jul	Jun	Jul	Jul	Jul	Jul	Jul	Jul	Jul	Jul	Aug	Aug	Jul	Aug	Aug	Aug	Jul	Aug	Aug	Aug	Sept	-
	Developm ends	KW	51	-	51	51	51	47	48	48	48	46	48	48	-	48	48	46	48	-	46	45	46	45	48	45	43
		Month	Dez	-	Dez	Dez	Dez	Nov	Nov	Nov	Nov	Nov	Nov	Nov	-	Nov	Nov	Nov	Nov	-	Nov	Nov	Nov	Nov	Nov	Nov	Okt
	DdR at 31.12.2014		**3,3**	2,3	**3,2**	**3,0**	**3,3**	**2,6**	**2,3**	**2,8**	**2,6**	**2,3**	**2,3**	**2,8**	1,5	**1,9**	**1,8**	**2,3**	**1,8**	1,2	**1,7**	**2,4**	**1,6**	**1,7**	**1,5**	**1,4**	0,7
	Nymphs / 100m²		163	230	187	46	374	73	245	167	44	8	169	121	196	25	45	73	68	166	22	46	43	82	4	29	0

Figure 4.17: Daily development rate (DdR) dependent indices for 2013 and 2014, calculated from soil temperature data (raw data with 10 min resolution). Month and calendar week (KW) of occurrence are denoted, if possible. The total sum of DdR based on complete annual datasets are depicted in bold black letters, whereas white letters stand for underestimated values due to raw data gaps (DdR at 31.12.). Data gaps can also inhibit the description of other indices (-). FB is the only site where development could not be completed (DdR < 1). Along with the incides, the total amount of sampled nymphs (Nymphs/100m²) are given for each year.

For example, the difference between development starting times for GH (992 m a.s.l.) was only one week and five weeks for AW (111 m a.s.l).

Interestingly, the time when **egg development is complete** (DdR = 1) varied from calendar week 28 – 36 in 2013 and, accordingly, from KW 25 – 36 in 2014. Again, the time differences between both years were larger at study sites of low altitude (two weeks for AW, zero for GH).

All study sites showed a more highly **total sum of egg development rates** at the end of the year 2014 than 2013, looking at sites with an complete microclimatic dataset in both years (bold black letters, DdR at 31.12). And, of course, again the differences looking at both years were larger at study sites of lower altitude (AL:16% and GH: 7% difference). The duration of development (time from start to end of development in weeks) was on average about 1.5 weeks longer in 2014 than in 2013.

Feldberg (FB) was the only site where egg development was never completed (DdR < 1) even in the warm season of 2014 and, in addition, the only site no ticks were found.

Egg development started earlier and reached more highly levels in 2014 at the site with southern sun exposure (**FR**), the clearing (**RF**) and a broad-leaved forest at high altitude (**DS**) compared to sites with no distinct exposure of similar or lower altitude (**FR:** FN, BT, KT, EP and **RF:** PH, FN, BT). However, the clearing also showed an earlier offset of sufficient development conditions.

In contrast, study sites with northern sun exposure (**CW, WR**), dense coniferous stands (**VS, AH**) and the gully habitat (**HQ**) show a delayed occurrence and lesser development progress in total.

Beside the latter, the **date** was calculated **until eggs would have to be laid** in the active season of the ticks (DdR = 0) to be able to complete their development until “development ends” (DdR = 1) (data not shown). The latest date was calculated for a riparian forest in the *Oberrheintiefland* (AL) in mid to end of August (2013: KW 33; 2014: KW 35). The shortest time span for a successful egg development occurred, as expected, at the highest study site with a stable tick population: Gosheim (GH). Eggs had to be laid until the beginning of July (2013 & 2014: KW 27) for a successful development.

In addition, the **total time span for a successful egg development** (duration from development start to the last time for egg laying, as described in the last paragraph) ranged in 2013 at minimum from 11 weeks (GH) to 18 weeks (AL) at maximum, and in 2014 from 12 weeks (GH) to 24 weeks (AL).

4.3.4 DISCUSSION AND CONCLUSIONS

NYMPHS, ALTITUDE AND ANNUAL AVERAGE TEMPERATURE

The results suggest that the factor temperature has its largest effect on tick densities at more highly altitudes with restricted temperature budget throughout the year. If the temperature is suitable for the tick's living processes, no restrictive effects should be noticable (see also explanations in Chapter 5.1.1) . The threshold of these process break-over was detected to be about 500 m a.s.l. and, accordingly, 8.5 – 9°C mean annual air and soil temperature. Of course, the image of a stripe would be more appropriate than the (threshold) line to separate the area where temperature is always sufficient from areas where temperature acts limiting for a tick's living processes, since temperature conditions can differ remarkably in space and time (depending on the weather situation in a year). For example, tick populations on the south facing slope of a mountain experience more warmth in the course of a year than those at the opposite side. The effect of this situation was examined by Cadenas et al. (2007) in Switzerland. They found that the questing tick density was more highly at the south facing-slope compared to the northern-slope and that the tick density on the southern slope was increased at more highly altitudes compared to results from a previous study at the same location – attributed to an increased summer temperature. These findings therefore confirm the image of a stripe on the one hand and the positive effect of temperature on tick abundances on altitudes with a limited heat budget on the other hand.

Regarding the results of this thesis, low tick densities seem to be typical for areas with restricted temperature budget, in accordance with findings in other study regions at high altitude (Jouda et al. 2004, Materna et al. 2008). In contrast, areas with suitable habitat type and temperature conditions seem to promote medium to high tick densities and low tick densities are an exception, in agreement with findings by Schulz et al. (2014) in southern Germany. All of the methods used – visual analysis, different regression model approaches and cluster analysis - lead to comparable results, all supporting the latter statements. I therefore assume that this temperature threshold describes a break between areas with

(1) a strong and subordinate effect on ticks' living processes and thus abundances as a limiting factor

(2) areas where temperature is not a limiting factor, but can influence ticks short-term behavior (by increasing the saturation deficit – in (Perret 2003)) and increase mortality by extreme events (Dautel & Knülle 1997, Dautel 2010).

Concluding, temperature seems to be the most important factor which has to be considered first when characterizing a study region, to be able to interprete results correctly.

It has to be stated that this kind of temperature margin is now decribed for the first time. Hence, no further literature exists to integrate the results into a greater context. Hopefully, these findings will motivate other Europeaen researchers to indicate altitude and mean annual temperature as important characteristics of the study site in the future. Many publications have very interesting outcomes, but lack information to classify and compare them with results of other regions (for example Korotkov et al. (2015) and Martello et al. (2014)). By providing information on altitude and mean annual temperature in the future we could be able to create a pioneering insight into the actual Ixodes ricinus population situation over Europe on the basis of highly comparable data.

HOW TEMPERATURE MARGINS CONSTRAIN FURTHER ANALYSIS

As to that, the detected negative influence of coniferous forests on nymphal densities could be just an artifact or at least overlapped by the temperature-dependent altitude effect, because all coniferous forests in this study are located at altitudes more highly than 500 m a.s.l. This conclusion is supported by results by Schulz et al. (2014) in southern Germany, where sampled tick densities of *I.ricinus* in coniferous forests were more or less similar compared to those in broad-leaved forests which were all located at low altitudes (204 to 508 m a.s.l.). Therefore, ignoring the effect of temperature would have led to the statistically verifiable but most likely false assumption that coniferous forests are in general less suitable habitats than mixed or broad-leaved forests in Baden-Württemberg.

Lauterbach et al. (2013) suggested that not the forest type (as a simple to use and easy to evaluate characteristic) but rather forest management practices and weather conditions (especially in winter) strongly influence dynamics of tick populations. However, results of this study indicate that coniferous forests at higher altudes and of low fragmentation (in accordance with results by Randolph (2001) and Kiffner et al. (2010)), which are dominant at the mountainous *Schwarzwald,* have a constantly negative influence on tick abundances whereas broad-leaved and loose mixed stands at the same altitude have not.

Therefore, I follow the statement of Medlock et al. (2013) that the ticks` habitat suitability diversify across Europe and add that, even so, specific forest types (here: pure and dense coniferous *forests in Schwarzwald) can have a constant influence on tick abundances on a local scale. Major causes for this are presented and discussed in detail in Chapter 4.5.2 and 4.5.3.3. In addition, these findings highlight the importance of field research to identify suitable habitats in a specific regional setting and narrow expectations of large-scaled, easily reducable factors.*

PREDICTION OF TICK MORTALITY IN WINTER

If tick abundances decrease strongly from one year to another, or are much lower as expected, a high variety of causes can be responsible for this. Indeed, abundances of larvae and nymphs decreased strongly within the time of investigation, from the year 2013 to 2014. One cause for this could have been the very dry (but for local thunderstroms with heavy rainfalls) summer leading to tick dessication in 2013 or unsuitable temperature conditions in the wintertime. Only mortality factors of the winter are analyzed, because the actual moisture conditions ticks were exposed to in summer 2013 are hard to predict, since ticks rest in shelter near the ground (Randolph 2004), where the humidity can be totally different to that measured in 50 cm height and those litter data were measured only at five Intensive Sites (compare results in Chapter 4.4.2).

Results lack the evidence that ongoing cold conditions below -10°C (Dautel & Knülle 1997) or even frequently occurring temperature changes as described by Herrmann & Gern (2013) caused the decrease of ticks. Furthermore, it seems unrealistic that such events could cause a decrease of ticks all over the country at all, because altitudes range from 100 m to almost 1.400 m a.s.l., so regions are included that normally provide moderate conditions, such as the *Oberrheintiefland* with its Mediterranean-like climate. Moreover, 95% of *I.ricinus* larvae (and most likely also the other stages) have been found to bury themselves in the upper soil for overwintering (Dusbabek et al. 1971) where recorded minimum temperatures stayed over the freezing point at all study sites over the entire winter.

Many days were detected with a high probability of frost in the litter layer due to freezing temperatures in the broad-leaved forest at Drackenstein (DS), but very little to none at the three study sites located in or near the *Oberrheintiefland*. The main period for this frost type is in late winter (February, March), when the soil beneath is cooled down to a certain extent. A minor thermal transfer from the ground to the litter zone above could promote freezing in the litter and upper soil layer where ticks rest in winter. However, how these 35 days of potential litter frost actually influenced tick mortality is difficult to say, because laboratory results were linked to freezing temperatures at -6°C (Dautel & Knülle 1997) and an actual frost occurrence depends also on a sufficient amount of stored water within the litter body (Sahlén & Goulet 2002).

Nonetheless, results do actually indicate that mortality caused by inoculative freezing is a problem limited to ticks at more highly altitudes, especially if overwintering ticks are not sheltered against temperature drops or temperature losses due to radiation processes in the litter and upper soil layer (Geiger et al. 2009, Geiger 2013). This therefore especially concerns 1) broad-leaved (-rich) forests because of their missing canopy coverage function and 2) areas with a low precipitation in winter and a resulting short-time and little snow coverage. In addition, the sheltering function of snow coverage can be partly substituted by the humus layer (Sahlén & Goulet 2002, Geiger et al. 2009), its insulating function increasing along with its depth (Putuhena & Cordery 1996). This could be an explanation why temperatures below the freezing point were observed in Auwald (AW) with a thin mull type humus but none at the close-by study sites Hardtwald (HW) and Michaelsberg (MB) with a medium dense, beech dominated moder to raw humus.

The effect of snow cover on tick mortality is one part of tick research that has not gained much attention yet. Until now, mainly positive aspects like the sheltering effect that preserve ticks from freezing temperatures have been discussed (Dautel 2010). In the opinion of the author, long lasting snow coverage likely intensifies temperature related problems at more highly altitudes, since it delays the start of tick development and activity in spring. Beyond that, melting snow could cause a more highly death probability due to drowning of ticks. Since the approach of this thesis is not suitable to test the latter hypothesis, it remains a task for future reserach.

To conclude, there was no evidence that conditions in winter 2013/2014 could have caused the observed reduction in tick abundances all over Baden-Württemberg, which indicates that other factors or weather events were responsible. In general, it seems that tick mortality caused by cold conditions is more likely at more highly altitudes combined with a broad-leaved forest type lacking an insulating canopy. However, to determine the actual mortality of ticks not only fine-scaled microclimatic data is needed but also data on the proportion of surviving ticks in winter rather than monthly data from March to October. An approach to measure tick mortality is described, e.g., in Dusbabek et al. (1971) and Daniel et al. (1977).

PREDICTION OF TEMPERATURE DEPENDENT DEVELOPMENT RATES

The results of the seasonal variation of development were comparable to those described in (Randolph et al. 2002), with highest development rates in summer due to warmest conditions and no development at all in winter, when temperatures fall below the threshold. However, the purpose of this approach was to characterize the suitability of the study sites for development of *I.ricinus* on the example of egg-to-larvae development, whereas Randolph et al. (2002) used development rates to interprete the seasonal activity of the tick.

The idea is that a successful egg development is the basis for every stable tick population and if conditions are suitable for eggs they should also be suitable for the development of every following stage. The results of the calculation coincide very well with results of tick sampling. At all study sites with a successful development (sum of development rates > 1), a stable tick population was found and, in addition, the only study site Feldberg (FB) without evidence for ticks was the only site where development could not be completed within a year.

Results of development rate dependent indices indicate that:

First, development conditions are bilateral restricted with growing altitude since the phase of potential development begins later and ends earlier within the activity season. Thus, the chance of a successful development and the chance to find a host is reduced for ticks in spring and autumn, compared to study sites in areas of low altitude. Together with findings by Materna et al. (2008), that the proportion of hatching eggs decline with increasing altitude, the observed decrease of tick abundances with altitude (see also Daniel 1993, Jouda et al. 2004) is explained very well. No limiting effect could be detected for lower study sites (DdR > 2). Thus, the results support the findings of the first part of this chapter which found a boundary that separates areas where temperature acts as a limiting factor and areas of sufficient warmth.

Second, the duration and starting time of the development phase is more weather dependent and thus highly variable at lower altitudes. In contrast, development conditions at high altitudes remain more or less constant and, in addition, unaffected by an early start of spring. These findings could explain results by Jouda et al. (2004), who found that the phenology of ticks were stable over the years only at high altitudes.

It has to be noted, that there is an important restriction for the interpretation of the results: The end of egg development (DdR = 1) does not automatically represent the date when larvae actually hatch. The cause for this is the so called morphogenetic diapause, an adaption of ticks which enables them to delay the time of moulting to obtain suitable seasonal conditions (Dautel

2010). In addition, the calculation of egg development started in spring after the temperature threshold was exceeded so that results refer only to eggs laid just before the start of development or for undeveloped overwintering eggs.

Results indicate that: If egg development remains uncompleted within one year, ticks are very likely absent in this area. Thus, using this method allows researchers to approximate temperature-dependent distribution boundaries for Ixodes ricinus for every northern or mountainous study region in the future. Even more importantly, the approximation by development rates would be much more precise compared to the factor altitude or mean annual temperature (as discussed in Chapter 4.1.3), even if they are based on data from nearby weather stations.

4.4 HUMIDITY AND TICK DENSITIES

Beside the temperature, the humidity availability is another crucial factor for tick survival. In the late eighties, Olaf Kahl performed pioneering work on the ability of *Ixodes ricinus* to absorb water from an unsaturated atmosphere. Ticks were able to do so if the relative humidity (RH) exceeded about 85% (Kahl 1989). In addition, since Perret et al. (2000) published their results on the negative influence of an increasing saturation deficit (SD) on questing tick activity, this factor was integrated in further research.

However, there are just few publications that measured climatic factors within a forest stand to evaluate their influence on ticks and, to the knowledge of the author, none that comprised such a high number of study sites and variety on measuring spots at the same time.

At first, it was tested whether any influence of climatic parameter on tick densities at an annual or seasonal time scale is detectable using two different approaches; resulting problems are discussed.

The second part determines the variability of climatic conditions on the example of the Intensive Site Auwald with a focus on the relative humidity factor and in respect to tick physiology.

The purpose of the chapter is to evaluate if moisture is a parameter that influences ticks on larger tempo-spatial scale and to show that data from official weather stations are not representative for actual moisture conditions ticks are exposed to in a forest habitat.

4.4.1 RELATIONSHIPS ON AN ANNUAL TIME SCALE

Figure 4.18 visualizes pairwise relationships between microclimatic parameters and nymphal densities on an annual time scale, as well as corresponding correlation coefficients.

Annual nymphal densities correlate strongly with temperature (positive) and altitude (negative) (as seen before in Chapter 4.3) and weakly with relative humidity and saturation deficit, especially in 2013. Contrary to expectations, relative humidity correlated negatively and saturation deficit positively with nymphal densities (the drier the more ticks).

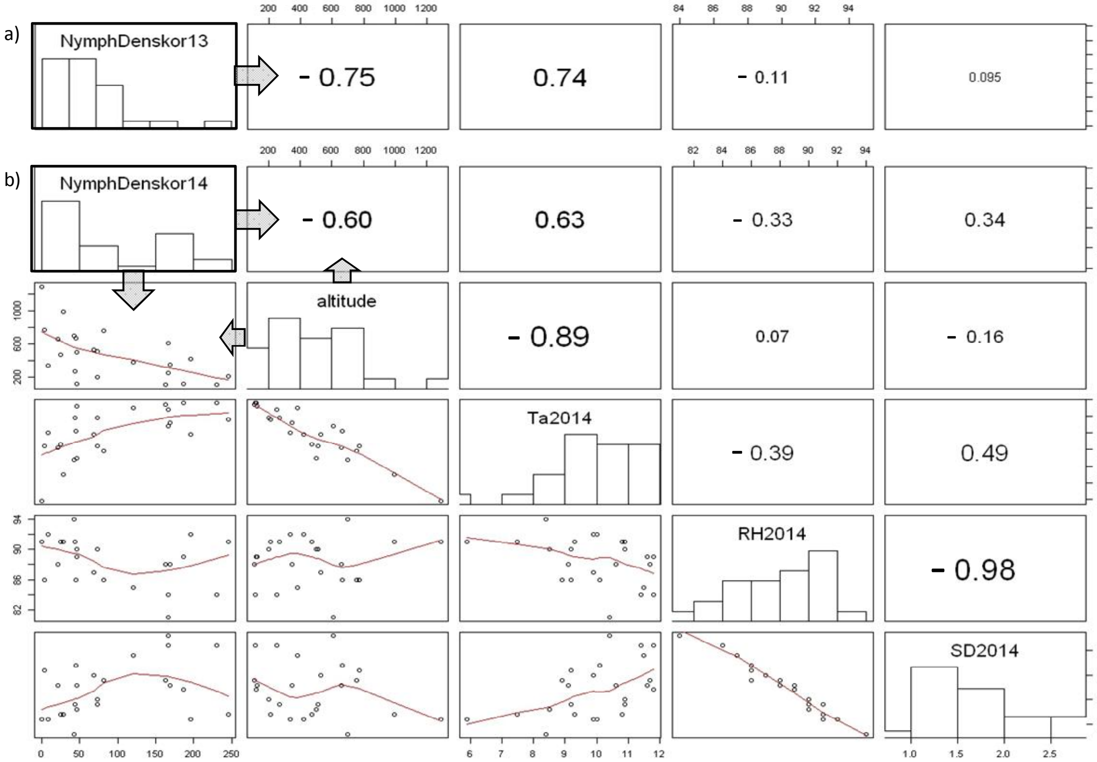


Figure 4.18: Scatterplot matrix with Spearman's correlation coefficients (upper triangle), histograms (diagonal) and LOWESS smoothed lines (in red, lower triangle) of nymphal density (NymphDenskor), altitude, mean annual: air temperature (Ta), relative humidity (RH) and saturation deficit (SD) in the year 2014 (b) and, as a short version, in the year 2013 (a). Outliers were removed for operating. The full dataset leads to the following correlation coefficients (from left = altitude to right = SD) in 2013: - 0.46; 0.46; -0.17; 0.11 and 2014: -0.58; 0.63; -0.29; 0.34). As an example, arrows depict the relation between nymphal density and altitude.

Furthermore, the correlation strength between nymphal densities and the environmental parameters decreased remarkably when the strongest outlier per year (BT in 2013 and AL in 2014) was included in the datasets outlier: Chapter 4.1.2.3).

In accordance with physical laws, the environmental parameter altitude correlates strongly negatively with the mean annual temperature, and the annual average of relative humidity with the saturation deficit. However, expected correlations between the temperature factor and humidity factors were less distinctive.

Table 4.8: Explanatory variables left in the reduced model. Data sets contain all study sites (1, 4 and 6) or are reduced by outliers (2,3 and 5,6). In no. 6, the explanatory variable vegetation period (Veg.ped.) is added.

		data set	**positive**	**negative**
2013	**1**	**full data**	SDspring**	SD.
	2	**minus BT**	$T_A$spring** RHspring	-
	3	**minus BT/ST/FN**	T_A** $T_S$spring*	altitude* T_S* $T_A$spring*
2014	**4**	**full data**	T_S** RHspring	RH** SD* $T_S$spring*
	5	**minus AL**	SDspring*** RHspring***	RH** SD**
	6	**plus Veg.ped**	T_S**	RH* SD* $T_S$spring* Veg.ped

The second approach used to identify parameters with an impact on tick densities was a backward stepwise regression. The different input datasets lead to various combinations of remaining explanatory variables in the reduced regression output as illustrated in Table 4.8.

The reduced models show strong differences in the character of remaining explanatory variables. For example, the model indicates a significant negative influences of mean annual RH and the antagonistic acting SD in 2014 (No.4) on nymphal densities at the same time.

4.4.2 COMPARISON OF CLIMATIC CONDITIONS AT DIFFERENT SPOTS

Up to now, moisture (and temperature) data of the Basic datasets were examined for impacts on and relationships with tick densities. The following chapter presents the complete microclimatic dataset of the Intensive Sites.

First, hourly averaged data were used to evaluate the variability of microclimatic conditions found within and beside the forest habitat and, additionally, to compare those with data from a nearby official weather station if possible. Figure 4.19, Figure 4.20 and Figure 4.21 illustrate the results on the example of the riparian forest Auwald (AW). Used data are hourly averages for the total period of investigation (Sep 2012 to Jan 2015). Descriptive statistics of the latter as well as the number of missing values are given separately for 2013 and 2014 in Appendix (C4).

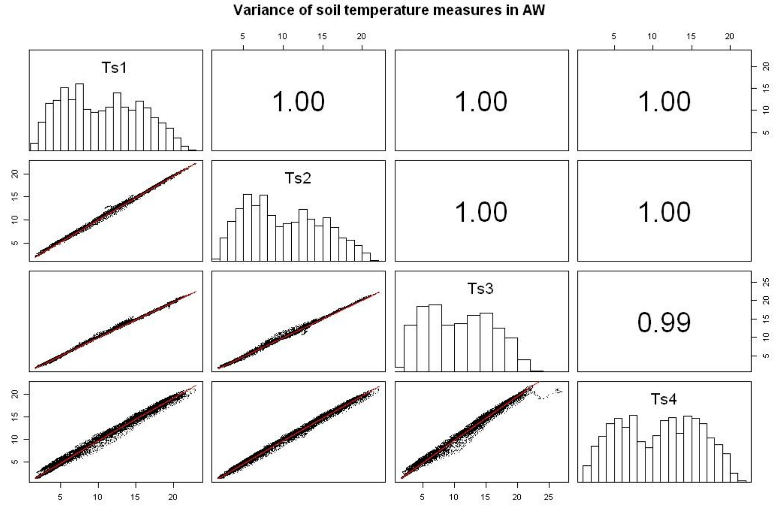


Figure 4.19: Scatterplot matrix for hourly mean values of soil temperature data from different spots in 5 cm depth (Ts1 to Ts4) within the riparian forest Auwald (AW) from 25th October 2012 to 1st January 2015.

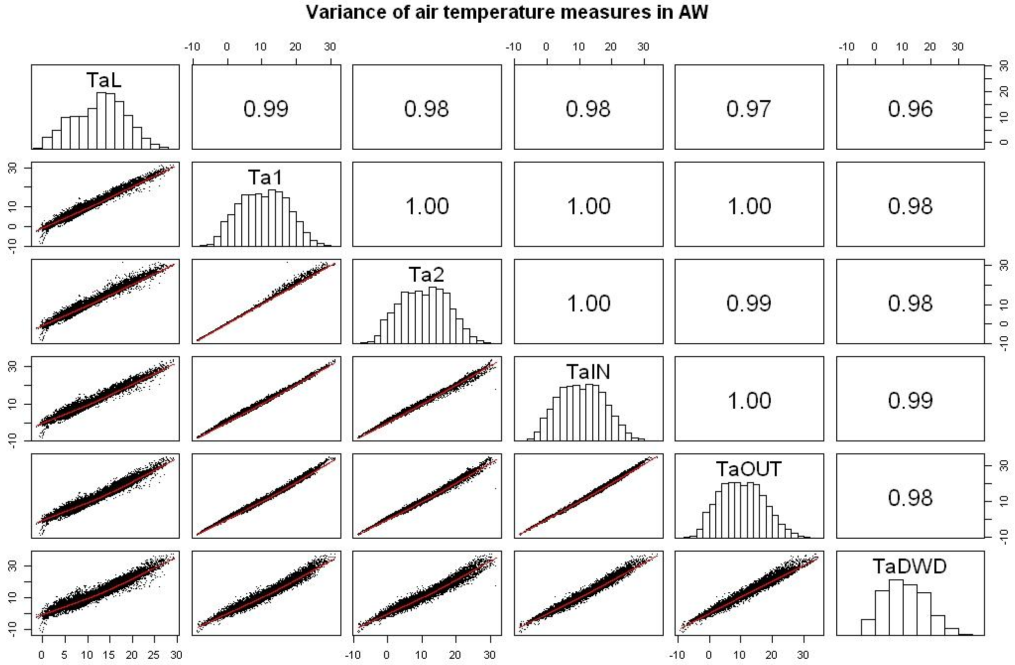


Figure 4.20: Scatterplot matrix for hourly mean temperature values in the litter (TaL), 50 cm height (Ta1 and 2), 2m height inside (TaIN) and outside (TaOUT) the forest Auwald (AW) and of a nearby official weather station (TaDWD, representing open land conditions in 2m height) from 25th October 2012 to 1st January 2015.

Four sensors measured the **soil temperature** at different spots at 5 cm depth inside the forests (Figure 4.19, Appendix (C4)). The results show little differences (2014, mean: < 0.5°C; r = 1.00 to 0.99) between the soil temperature conditions at different spots, even for maximum and minimum values (Min: 1.8 to 2.6°C, Max: 21.1 to 23.0°C).

Air temperature data cover three domains within the forest (litter, 50 cm and 2 m height) as well as the situation directly beside the forest (Figure 4.20). Hourly mean values of these domains and data from a nearby official weather station are highly correlated (r = 1.00 to 0.96). This is best explained by constant temperature exchanges from warm to cold which equalize temperature differences, i.e. sensible heat fluxes, following Lee 1978. However, maximum and minimum values differed considerably between different spots. As expected, highest and lowest values were measured outside the forest and at the official weather station (2014: 34.6°/36.5 °C and -9.0°/-12°C, respectively) whereas most moderate conditions were found in the leaf litter (2014: -1.4 to 28.0°C) according to results of the descriptive statistics (Appendix (C4)). T

he average temperature of all domains was comparable (11.6° to 12.2°C). In contrast, **relative humidity** differs to a much higher extent in correlation strength (r = 0.99 to 0.52) of data from different measurement-domains (Figure 4.21) than temperature data. The highest correlation was recorded between the sensors located in the herb layer (50 cm height). In addition, data measured in the free atmosphere inside and outside the forest (dona-ted as RHa: 1/2/IN/OUT) were all highly correlated (r = 0.99 to 0.96) to each other. Data of the nearby weather station (RHDWD), however, were less correlated to the latter (r = 0.89 to 0.80). Again, the strongest discrepancies were found for the litter layer compared to all other spots (r = 0.63 to 0.52).

Scatterplots of the official weather station (RHDWD) and, to a lesser extent, of the data measured at 2 m height (RHaIN, RHaOUT) show a characteristic deviation from linearity, forming a belly to minor relative humidity values. In contrast, scatters from data of the litter layer (RHL) concentrate on relative humidity values from 90 to 100%. These specific findings are analyzed and illustrated in detail in Chapter 4.5.3.

The annual average **relative humidity** shows a characteristic **increase from outside to inside the forest** and **from atmosphere to the ground** within the forest at all study sites, looking at main statistics (mean, median, max, min, see Appendix (C4)). The difference between the two zones marking the very end of the range (litter zone and outside) is in between 17 and 28% RH (mean: 22%) and the minimum values are considerably more highly (9-33% RH, mean: 24% RH) in the litter zone compared to all other zones.

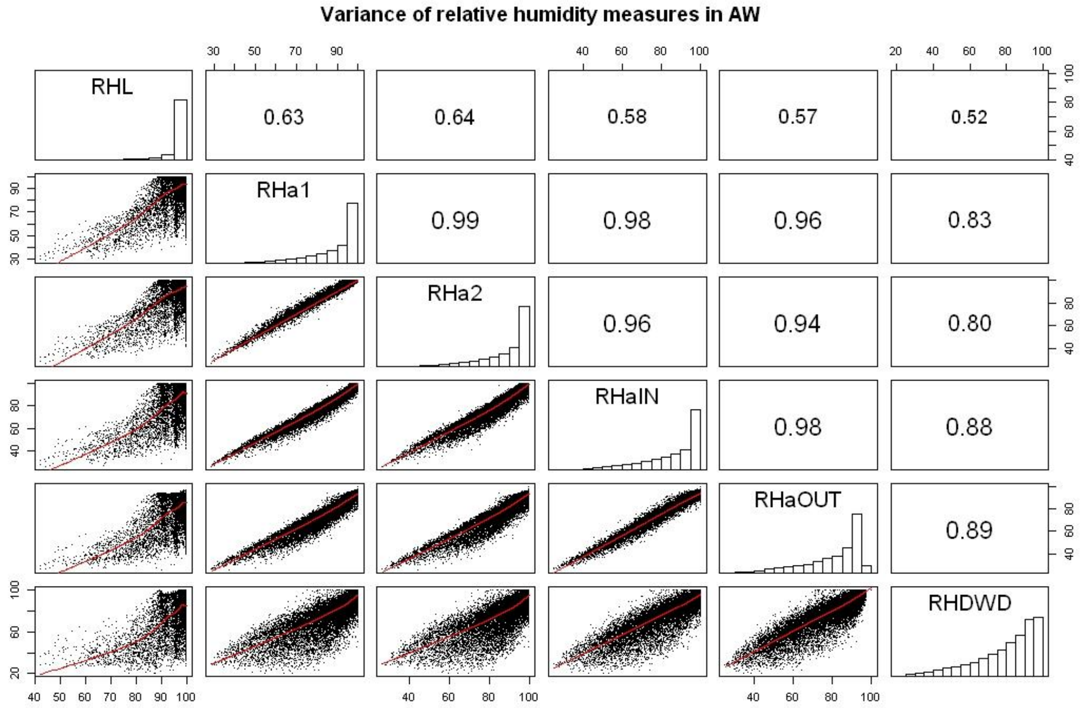


Figure 4.21: Scatterplot matrix of hourly relative humidity values in the litter (RHL), 50 cm height (RHa1 and 2), 2m height inside (RHaIN) and outside (RHaOUT) the forest Auwald (AW) and of a nearby official weather station (RHDWD) from 25th October 2012 to 1st January 2015.

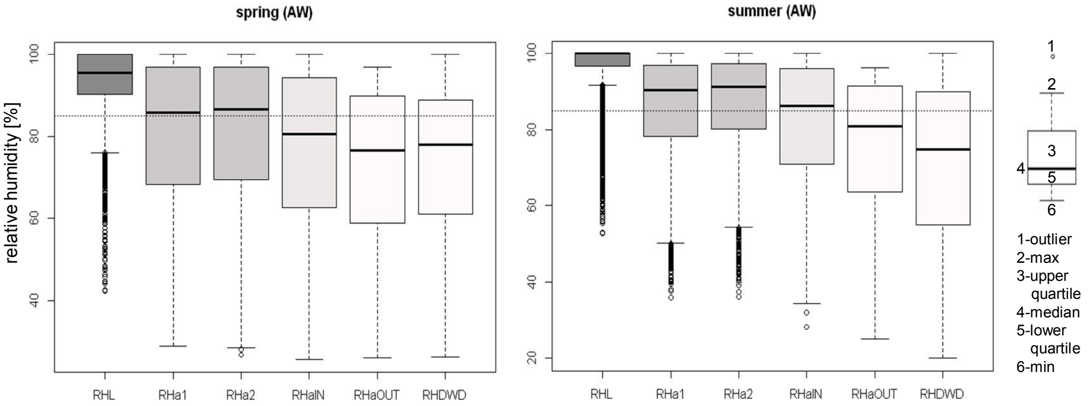


Figure 4.22 Hourly mean values of relative humidity in the litter (RHL), 50 cm height (RHa1 and 2), 2m height inside (RHaIN) and outside (RHaOUT) the forest Auwald (AW) and of a nearby official weather station (RHDWD) in spring (left, March to May) and summer (right, June to August) in the years 2013 and 2014. The horizontal dotted line depicts the critical equilibrium humidity for *I. ricinus* of about 85% RH (Kahl 1989).

The relative humidity in the litter zone stays at a high level most of the time, even if the atmospheric humidity drops. To illustrate these findings, the distribution of relative humidity (data: hourly mean) is depicted in Figure 4.22 for spring- and summertime in 2013 and 2014.

Conditions in the **litter layer** (RHL) stayed at a level where ticks are able to regain water losses (= over the dotted, horizontal line) most of the time in spring and, even more so, in summer.

In the zone of active tick questing (**50cm height**, labeled as RHa1 and RHa2), humidity conditions stayed at sufficient, undehydrating levels half of the time in spring and about two-quarter of time in summer.

With increasing distance to the forest ground (RHaIN, 2m), or, even more, to the forest itself (RHaOUT, RHDWD), the humidity conditions get more dry. Outside the forest, relative humidity rarely reaches levels over 90 %.

4.4.3 DISCUSSION AND CONCLUSIONS

RELATIONSHIPS ON AN ANNUAL TIME SCALE

Results from the scatter plot matrix showed that data from relative humidity measures are negatively correlated with those from saturation deficit and air temperature, in conformity with basic physical principles. While the environmental factors show no deviations from common knowledge, it is surprising that in both years a negative influence of relative humidity and a positive one for the saturation deficit on nymphal densities was detected. Since ticks are strongly dependent on sufficient moisture conditions, a negative influence of increasing relative humidity seems paradoxical. One way to explain these results is that the strong positive influence of temperature on tick densities covers potential causal relationships of ticks with relative humidity and saturation deficit. In other words, the negative influence of relative humidity on tick densities is just an expression of the physical relationship: rising temperatures – falling relative humidity; the positive influence of the SD an expression of: rising temperatures – rising saturation deficit.

Results from the backward stepwise regression seem similarly distorted. For example, the spring values of the antagonistic parameters RH and SD both had a positive influence on nymphal densities (model No.5, 2014). One problem that can distort the interpretations of regression analyses is collinearity: If two explanatory variables strongly correlate with each other and both have a remarkable effect on the dependent variable, the calculation process identifies only one of them. As a result, strength and, even worse, the direction of the relationship can be misinterpreted (Dormann & Kühn 2011). To avoid collinearity, explanatory variables must be analyzed singularly, since all measured parameters are temperature related. This throws the interpretation back to a simple linear regression or a correlation between a pair of variables, as illustrated in the scatter plot matrices.

In addition, it was striking that removing outliers had a strong effect on results. The correlation strength increased remarkably, when using scatter plots and, in addition, the relationship between explanatory variables and ticks changed their direction oppositional for the same year in the regression analysis. This is most likely an effect of undersized sampling sites. Research that is focused on the impact of environmental factors on tick presence on a spatial scale comparable to Baden-Württemberg is often done with a hundred sampling sites or more (Merler et al. 1996, Guerra et al. 2002, Eisen et al. 2006).

THE VARIABILITY OF HUMIDITY IN FORESTS

The findings agree with other studies related to the (micro)climate within forest stands.

Temperature extremes are reduced in the forest, especially in the leaf litter, compared to spots with an open field character (Scheffers et al. 2014). The moisture factor relative humidity was found to be highest on the ground and decreased along with growing distance, which can be explained by several processes leading to a more highly water vapor such as the storage of water in the humus, evaporation, plant transpiration and reduced mixing of air masses (Geiger et al. 2009).

Scatter plot matrices illustrate well that temperature data from the official weather station differ little from those measured in the forest, but relative humidity data differ strongly – especially from those measured in the litter layer and the herbal layer in 50 cm height. Since ticks live only in these two domains near the forest floor, data from official weather stations are unsuitable to describe moisture conditions in a ticks habitat. These findings support demands by Estrada-Pena et al. (2013) on tick researchers to avoid the use of official weather data to explain tick activity pattern. Instead, they encourage researchers to gather their own climatic data as done in our approach. However, looking at the results of the last chapter, it seems realistic that official weather data can be used to estimate the temperature dependent suitability of a habitat on a larger spatio-temporal scale, which means a enormous reduce in costs and efforts.

Results indicate, that temperature conditions in the leaf litter, and even more so, in the upper soil layer, stayed within the physiological tolerance of *Ixodes ricinus* all the time. In times near maximum temperatures (i.e. over 20°C for immature stages), an increased stress activity of ticks is realistic (Sixl & Nosek 1971), which could lead to an avoiding behaviour that leads them to cooler conditions in the upper soil, given that the soil provides sufficient interstitial conditions (Merler et al. 1996, Schwarz et al. 2009, Petney et al. 2013). To give a study site related context: In the very hot and dry period in the summer of 2015, most soils were highly desiccated even in forests. Especially substrates with a high portion of clay shrunk to a compact mass, broken by deep cracks, with hardly any interstitial room left. This situation is expected to led to a very high mortality of ticks in habitats with a clay rich soil which should be visible in tick densities and cases of tick-borne diseases in 2016.

The humidity conditions in the leaf litter layer turned out to be very sufficient for ticks, staying most of the time within the range where ticks can maintain their water balance (Kahl 1989, Gaede & Knülle 1997), even in summer. These findings support former statements of other researchers that the leaf litter provides shelter and suitable moisture conditions for ticks (Gassner 2010, Schulz et al. 2014). This statement is commonly used to explain findings according to tick densities and activity pattern in general, but hasn`t been proven yet in such detail.

Highly opposing conclusions would be made looking at official weather data, because the recorded dry conditions would mostly lead to a desication of ticks (Kahl 1989), especially in summer (illustrated in Figure 4.22). High mortality rates would be expected (reviewed in Randolph 2004), although ticks hiding in the leaf litter or even actively questing in upper parts of the vegetation were well provided with moisture.

It is somehow surprising that relative humidity conditions were more moist in summer compared to springtime, especially because more highly temperatures naturally reduce relative humidity values (Zmarsly et al. 1999). Concluding, the absolute humidity must have been much more highly in summer compared to spring in both years. A more highly absolute humidity is likely a matter of an increased precipitation amount, which accords with the long-term averaged precipitation distribution in Baden-Württemberg where a considerably more highly amount of precipitation falls in summer (especially in June and July) compared to spring (DWD & LUBW 2006).

A major issue hasn`t been addressed yet: Why is there no relationship between annual scaled humidity data measured directly in the ticks habitat and the related tick density?

I would explain this as follows: on average, the humidity of forests in Baden-Württemberg turned out to be very moist with values over 81 % measured in 50 cm height in both years. In addition, data from the litter layer showed that conditions are even moister and more balanced in this domain, which leads to the assumption that moisture conditions should be – in general – suitable for survival (see also Medlock et al. (2013)). High tick mortality is therefore expected to be a matter of temporarily very dry and hot periods or of unsuitable characteristics of the humus type and herbal coverage (following results of the next chapter). It seems, that averaged data can not represent those factors and that several factors related to the moisture availaibility in the ticks` microhabitat have to be considered simultaneously to estimate its suitability.

Consequently, the next chapter analyses the suitability of ticks`microhabitats near the forest floor in relation to several vegetational characteristics, especially stand density and humus type, in different situations over a tick activity season.

4.5 DECONSTRUCTING FOREST-MICROHABITATS

In the last chapters, results indicated a major influence of the temperature factor on tick densities but none for the relative humidity, on the examined temporal scale. Moreover, relative humidity turned out to be a factor with a high tempo-spatial variability within a forest stand.

To complete the statistical analysis of environmental factors, vegetational parameters are tested for a detectable impact on tick densities in the first part of this chapter .

In tick research, simple descriptive and test statistics are often used to examine the relationships between ticks and their environment (Schwarz et al. 2009, Boyard et al. 2011). However, relationships with vegetational parameters are very likely a matter of the microclimate created by plants and their remnants and its support for tick survival, naturally highly complex and thus hard to determine by simple statistics.

The mechanistic understanding of underlying processes within the ticks' microhabitats is therefore crucial to interpret results in an appropriate way (see also Lehman (1986), Levin (1992)), especially with regard to the highly variable relative humidity factor. Thus, the following part examines how specific vegetational characteristics are to be interpreted concerning ticks' living-processes by deconstructing them to fundamentals: temperature and relative humidity, on a habitat-scale.

The hypothesis that forest stand density is an important factor for tick development in areas of limiting temperature conditions is evaluated in the second part of this chapter, using supplemental data from tick samplings and temperature measurements in the litter layer at a study site located at high altitude.

In the third part, microclimatic differences between and characteristics of two major humus types (thick and continuous versus thin and temporarily) are analyzed along with possible consequences for inhabiting tick populations.

The last part examines the context of precipitation income, soil moisture as well as storage capacity and moisture availability for ticks in different humus types.

4.5.1 PRELIMINARY STATISTICS

Prior to process-oriented analysis, a test for impacts of environmental factors on tick densities was performed. According to the nonparametric Kruskal-Wallis test the nymphal densities showed no significant differences if grouped after the parameters **crown density** and **herbage incidence,** and no differences at all in the year 2014.

Different medians between nymphal densities of all 25 study sites were detected in the year 2013 for the factors:

(1) Forest type: $p < 0.05$ *
(2) Litter depth: $p < 0.05$ * (& for study sites above 500 m a.s.l.: $p < 0.053$)
(3) Litter species: $p = 0.063$

Since those results can be scattered by temperature-dependent effects on tick densities (see Chapter 4.3.1.2), it seems necessary to examine the distribution of these significant factors within two zones of "low" and "high" altitude (</> 500 m a.s.l.). Feldberg is the only study site where ticks currently not occur, and thus separated from the latter groups. Figure 4.23 illustrates groups of study sites according to vegetation parameters (x-axis) and the nymphal densities found at those study sites in 2013 (y-axis), subdivided by different temperature zones (low = zone a; high = zone b; FB = zone c).

Forest type: The main part of study sites characterized by broad-leaved and mixed forests is located in zone a (low), whereas coniferous forests are restricted to more highly altitudes (zone b), as seen before in Chapter 4.3.1.2. **Litter species**: According the distribution of the forest type, the main part of sites with a leaf litter is located in zone a, and sites with a distinct needle litter are dominant in more highly altitudes. Especially study sites with beech dominated litter concentrate at lower altitudes. Sites with low **litter depth** concentrate in the lower zone a, while medium to high litter depth are more or less equally distributed over both zones a and b.

The distribution of study sites indicate that the results of the Kruskal-Wallis test for forest type and litter species are likely distorted by temperature-dependend effects. In contrast, the litter depth actually has an effect on nymphal densities. To explain this relationship, a process oriented analysis is carried out in Chapter 4.5.3.

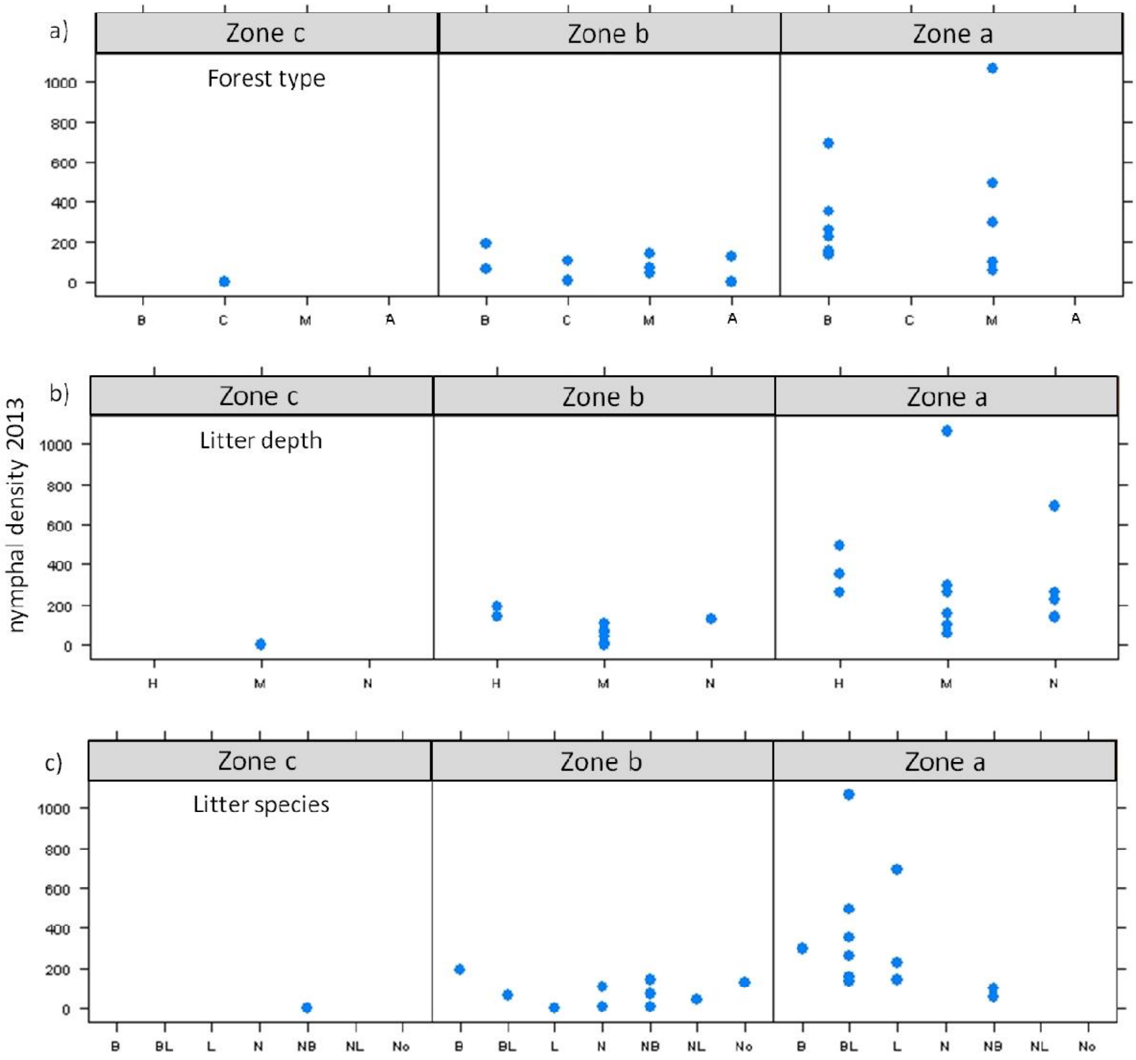


Figure 4.23: Nymphal densities (cor) found in 2013 grouped by forest type (B = broad-leaved, C = coniferous, M = mixed, A = others), litter depth (H = high, M = medium, N = low) and litter species (B = beech, L = non beech leaves, N = needles, no = no leaves nor needles). Study sites are subdivided by their temperature zone (Zone a = < 500 m a.s.l; Zone b = > 500 m a.s.l,; Zone c = Feldberg).

4.5.2 EFFECTS OF FOREST STAND DENSITY

Comparably high tick densities at the mountainous study site Gosheim in 2013 lead to the hypothesis that a thin stand density could have a positive effect on tick development at more highly altitudes (see method description in Chapter 3.2.3). As shown before, temperature likely acts limiting for tick development and activity there (Chapter 4.3.1). The hypothesis is based on the assumption that clearings offer warmer conditions due to the more highly amount of directly incoming solar radiation than constantly shadowed areas. Thus, thin stands should provide patches of warm conditions on the ground where development can be completed quite fast even at high altitudes.

4.5.2.1 TEMPERATURE CONDITIONS

To investigate the different conditions that are provided, additional litter temperature measures were carried out in spring and summer 2014 (2014-04-01 to 2014-08-31) at different spots within the manifold, coniferous rich mixed forest in Gosheim at an altitude of about 1000 m a.s.l.. Sensors took data directly on the mineral ground surface, embedded in the surrounding litter mass.

Two different logger configurations were operated (original and modified). First, Figure 4.24 presents results according to the **two logger configurations** as the temperature difference of simultaneously measured data, at two different spots (shadow and wide clearing).

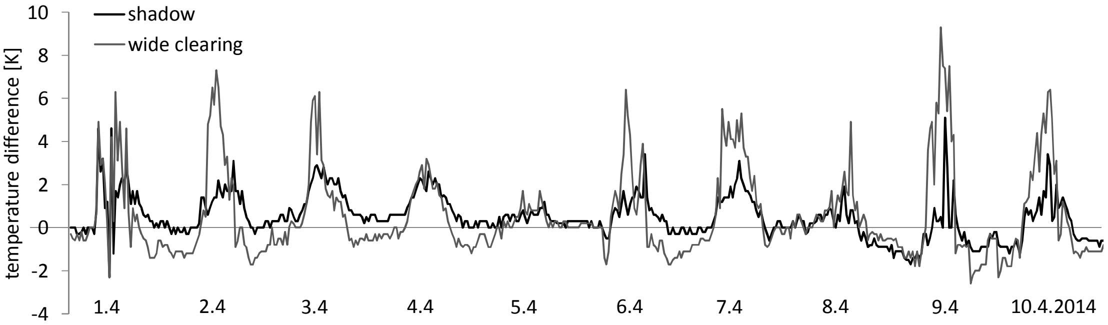


Figure 4.24: Comparison of the resulting temperature data measured with modified (external sensor) and original (internal s.) data logger. Both logger systems were operated together (1) within the moss layers under a huge coniferous tree (shadow) and (2) under grass, located at a wide clearing. Modified loggers always reached more highly values at day (peaks) compared to the original loggers.

Data from the different logger systems show two trends (Figure 4.24):

1) the modified loggers detect temperature peaks (min = night, max = day) better than the original loggers
2) differences between the loggers were greater at the clearing compared to the shadow

These trends were expected, since the sensors of the modified loggers are directly influenced by surrounding temperatures while the original loggers are not. The first trend can be explained by a direct energy uptake (= that ticks would experience) at the external sensor compared to the original logger set-up with an internal sensor recording temperature changes indirectly via a heat-conducting-paste. The original logger reacts more slowly, which would explain its lesser ability to detect short-time temperature peaks. The second trend presumably reflects a more highly amount of incoming solar radiation at day and a more highly amount of emitted longwave radiation at night at the clearing, compared to the sheltered spot (see Chapter 2.2.2). In addition, the temperature in the litter at the small clearing shows an interesting daily course: Until about 11 am, the temperature rises, then drops down constantly and reaches a temperature level similar to the other litter temperatures at about 3 pm. This can be explained by the shadowing effect of large trees bordering the clearing which causes a so called "horizon limitation". Indicated by the data, the incoming solar radiation reached the ground directly until about 11 am, afterwards the neighbouring tress provided shadow. Accordingly, temperatures decreased within the next hours due to heat exchange processes with surrounding, cooler bodies.

Concluding, the modified logger configuration represents the actual conditions in the litter/moss layer better than the original one. As a consequence, the following analysis of litter temperature conditions is based only on data measured by modified loggers, because these data are closer to the actual conditions developing ticks experience in the litter layer and results from litter measures are more comparable using the same logger type.

Beside these litter data, data exist that were measured in the soil and in 50 cm height within the forest (Basic Station) as well as data measured at a nearby official weather station. Comparable to the modified litter loggers, sensors of these loggers system are directly influenced by surrounding temperatures. Resulting data thus represent the actual temperature conditions of their spot very well. A comparison of these data leads to new insights about the variety of conditions ticks face within one forest, depending on where they are, and how well data from official stations, measured in the open field, can represent the conditions within the forest. Descriptive statistics (Table 4.9), time series of the total measuring period (Figure 4.25) as well as daily temperature courses for April and July (Figure 4.26) present the data. The analysis includes all areas where ticks likely occur (litter, 50 cm height) as well as borders (soil, 2m height) of those areas.

Table 4.9: Descriptive statistics of temperature data [°C] measured at Gosheim (GH) from 2015-04-01 to 2015-08-31: at the official weather station (2 m height, DWD), the Basic Station (BS) at 50 cm height (BS_Ta) and in the soil (BS_Ts) and at four different spots within the litter layer by modified logger systems (under grass: wide clearing; under moss: timberland, shadow, small clearing).

	DWD	**BS_Ta**	**BS_Ts**	**wide clearing**	**timberland**	**shadow**	**small clearing**
Min	**-1.8**	**-1.9**	**3.3**	**-3.0**	**-0.7**	**-0.1**	**-0.4**
1st Qu.	8.8	8.1	8.4	9.1	8.5	9.1	8.7
Median	12.4	11.5	11.5	13.3	11.3	12.5	11.9
Mean	**12.4**	**11.5**	**11.1**	**13.2**	**11.4**	**12.9**	**11.8**
3rd Qu.	15.5	14.7	13.7	16.4	14.1	15.5	14.7
Max	**30.3**	**31.4**	**16.9**	**41.9**	**30.4**	**53.5**	**33.4**
Stabw	5.2	5.0	3.1	6.3	4.2	6.0	4.6

The mean temperature of the **official weather station** (DWD, 2 m) data is very close to the averaged mean temperatures of all four litter spots (= 12.3°C), but differs strongly in its maximum extent. Air temperature data from the DWD in general match very well with those of the **Basic Station** (50 cm), especially in times of temperature drops (Figure 4.25). As expected, the **soil** temperature stayed cooler and showed a lesser value extent than all other measures located above. Temperature conditions measured within the **litter** layer differed weakly in minimum but strongly in maximum temperatures.

The following part compares conditions at different litter spots. As expected, the **timberland** was the coolest litter spot in average while the **wide clearing** was warmest. The order of spots regarding the highest to lowest daily mean values **change frequently** over the measuring period (see Figure 4.25, grey bars). Surprisingly, the highest maximum value (53.5°C, measured on 19th July) was recorded at the **shadow** spot under a large coniferous tree and its daily mean values exceeded frequently those of all others.

In addition, conditions at the edge of a **small clearing** were colder as expected, especially by comparison with the timberland. One approach to explain these findings is the different warming potential of the directly incoming solar radiation within a day and throughout the season, since the solar radiation is strongest if the sun is at or near its zenith: from noon to early afternoon and in summer. Thus, the warming of a specific spot at the ground depends strongly on shading effects from surrounding trees in the course of a day and a year. An example for changing conditions within the course of a season is the sha-dow spot (Figure 4.25, royal blue). It was relatively cold at the beginning of the season and developed to the place with the warmest conditions throughout the summer, which is likely caused by an intensified radiation and resulting warming in the course of a day.

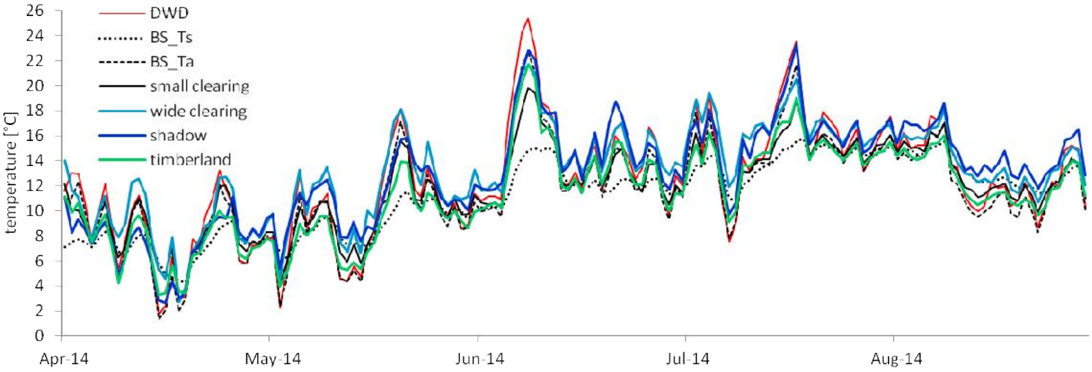


Figure 4.25: Time series of daily mean temperatures in the litter (solid lines in black and blue) as well as soil (Ts) and air (Ta) temperature at the Basic Station (dotted lines) measured at different spots in Gosheim (GH) from 2015-04-01 to 2015-08-31 combined with data (2 m height) from the nearby official weather station (red). Grey bars highlight times of distinct differences in the order of spots (warmest to coldest).

The logical next step is to examine the development of temperature conditions over a day, in different times of a season. In Figure 4.26, monthly averaged data are used to illustrate the character of the temperature course at each spot rather than giving an example of a specific day. It can be seen, that some spots gathered much more heat during the day than others and that the warming was either constant, forming a bell-shaped curve (wide clearance), or occurred at a specific daytime.In April (Figure 4.26., left), temperatures rose more highly in the litter located at the **wide clearing** compared to all other litter spots with a difference between the averaged daily maximum of about 6.2°C (1.8°C at the small cl. to 8.2°C in the timberland).

In both months, the warming at day follows a bell-shaped curve. The temperature in the litter at the **small clearing** (solid black line) shows an interesting daily course in April: Until about 11 am, the temperature rose to a large peak, then dropped down constantly and reached a temperature level similar to the other litter temperatures at about 3 pm.

In contrast, only two small peaks occurred in July (Figure 4.26, right). At the **shadow** spot, conditions (royal blue) even exceeded those at the wide clearing in July, showing two large peaks at about 10:30 am and 1:30 pm. Temperatures at **timberland** stayed at a low level even in summer, with mean temperatures of 7.4 °C (max = 10.5°C) in April and a mean of 14°C (max = 17°C) in July.

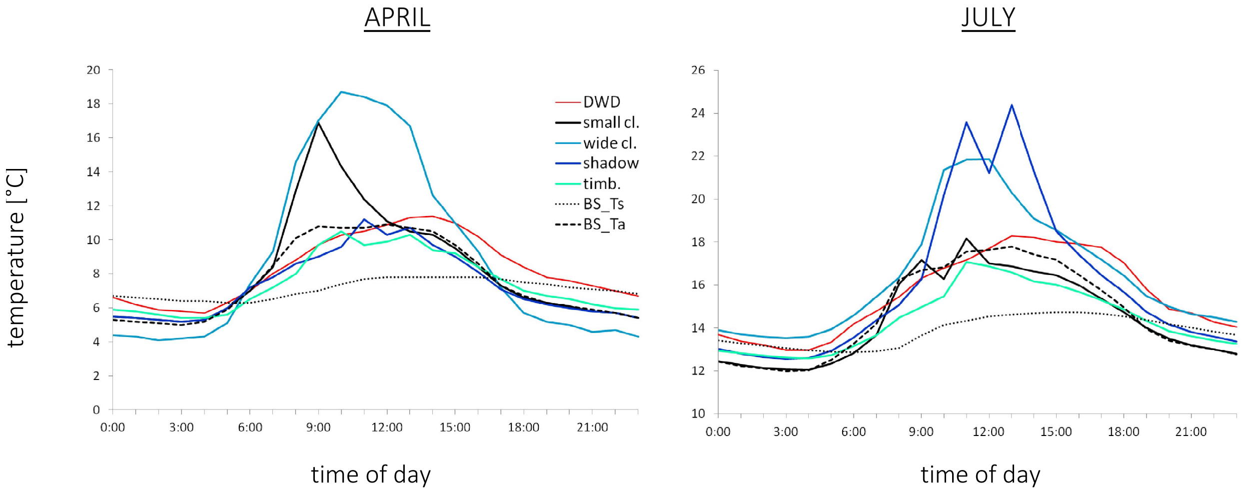


Figure 4.26: Daily temperature course at different spots at Gosheim. The hourly values are calculated as monthly averages of data in April (left) and July (right) 2014. The coldest conditions in the litter layer were recorded at timberland, the warmest at wide clearing and shadow (only July).

As expected, conditions measured in the air at 2m and 50 cm height were more moderate, forming a flat bell-shaped curve. Here, the warming at day is a result of the energy flow from the warmer ground surface to the air and the mixing of air parcels (see Chapter2.2.2 for background information). Comparable conditions were found at the litter spot in timberland. It showed no relevant short-term warming, which leads to the assumption that its diurnal warming is primarily based on the energy flow from the warmer atmosphere above because the sunlight is widely absorbed by the dense canopy so that temporarily sunspots are rare. In contrast, the sun is able to reach the ground and thus causes a local warming at all other litter spots. That leads to a constant warming if trees rarely shade the spot (wide clearing) or irregular temperature patterns if trees temporarily shade the spot (small clearing, shadow) during the day.

In summary, the data reflect very well the variety of temperature conditions to be found in a thin forest stand. The assumption that clearings offer warmer conditions in the litter layer than shaded areas was constantly true for the wide clearing and the timberland. Areas characterized by a frequent change of patches made of groups of trees (shadow) and small clearings showed a high diurnal and seasonal complexity in temperature conditions due to the changing shading effect of trees with the position of the sun. The last part of the next chapter gives an answer to the question how these differences affect the development of *I. ricinus* eggs on this temperature restricted study site at high altitude.

4.5.2.2 *IXODES RICINUS* ABUNDANCE AND DEVELOPMENT

Along with microclimatic measurements, ticks were sampled in different areas on 100 m² each to identify possible effects of temperature differences on questing tick densities. Results are given in Table 4.10, subdivided by life stages. Over the time of investigation, sampling captured exclusively ticks of the species *Ixodes ricinus*.

Table 4.10: Number of *Ixodes ricinus* ticks per 100 m², sampled at different forest parts at the study site Gosheim (GH) in 2014. The mixed stand comprises the temperature logger spots “shadow”, “small clearing” and the Basic Station.

	wide clearing			**timberland**			**mixed stand**		
	larvae	nymphs	adults	larvae	nymphs	adults	larvae	nymphs	adults
01.04.2014	0	2	1	0	0	0	4	0	0
16.04.2014	0	0	1	0	0	2	0	1	8
26.04.2014	0	1	5	0	1	0	0	0	2
06.06.2014	0	4	3	0	1	1	0	9	4
02.07.2014	0	2	1	0	2	0	0	1	1
16.07.2014	0	1	4	0	3	1	0	2	1
31.07.2014	0	0	0	0	0	0	0	0	2
21.08.2014	0	3	1	0	0	0	0	0	1
10.09.2014	0	0	0	0	0	0	0	0	2
	0	13	16	0	7	4	0	13	21

According to the non-parametric Kruskal-Wallis test no significant difference between the samplings were found. The highest difference was found between adults sampled in the timberland compared to the mixed stand ($p = 0.23$). However, it is noticeable that the number of ticks found at timberland (11) is lower than those at the other two spots (29 and 34)

.

Figure 4.27 visualizes the **activity course** of nymphs and adults throughout the spring and summer season 2014.

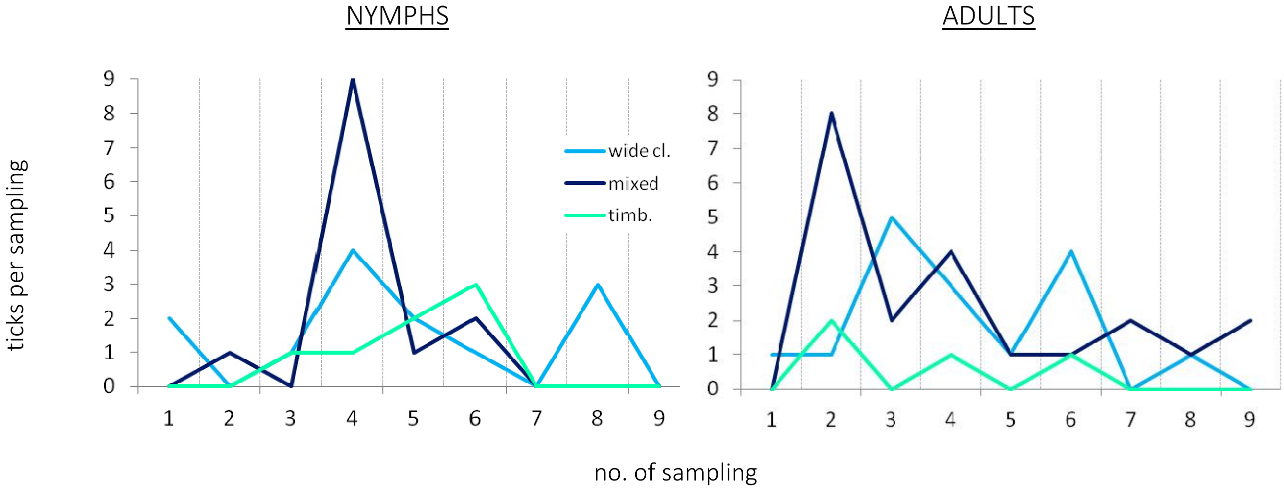


Figure 4.27: Occurrence of questing ticks per 100 m² in different forest parts in Gosheim, based on 9 samplings from 2014-04-01 to 2014-09-10. Numbers of ticks differed noticeable at different forest parts.

Most nymphs were active in June in the mixed stand and the wide clearing. After that, numbers stayed relatively low over summer.

It has to be mentioned that the weather was very wet in summer 2014. Consequently, the moist vegetation often soaked the sampling cloth and thus hindered tick sampling (JENSEN et al. 2000). Low numbers of ticks in summer therefore could be just an artifact of the sampling method itself. If it is no artifact, results indicate that **rainy weather** constricts the ticks questing activity. Certainly, rainy weather does constrict the adjacent interpretation of the data.

Besides the questing activity, the **development** of ticks is crucial to maintaining a stable population. Since larvae of *I. ricinus* were found in 2013, I assume that the tick population is stable at Gosheim and, accordingly, that ticks can develop successfully. Results from Chapter 4.3.3 already indicate that eggs are able to develop within one year, based on temperature data measured in the upper soil.

Since *I. ricinus* females lay their eggs often on the ground within the shelter of the surrounding litter or, in this case, of the moss body, the additional litter data should provide a good estimation of actual development conditions at different spots of the forest. Figure 4.28 illustrates results as the **sum of daily development rates**. Thereby, development is completed when the sum reaches the value 1 (DdR_{sum}= 1). Additionally, Table 4.11 lists these dates of development completion for all spots and provides a cross-analysis of the time differences, given in days .

Table 4.11: The cross table illustrates the time difference (in days) of the date when egg development is completed (DdRsum = 1) between different spots (for details see Table 4.9 and the Figure above) and domains (soil, air and litter).

		soil	*litter*				*air*	
DdRsum = 1		**SS_Ts**	**sm. cl.**	**wide cl.**	**shad.**	**timb.**	**SS_Ta**	**DWD**
03. Sep	**SS_Ts**	-	-25	-47	-41	-15	-26	-37
05. Aug	**sm. cl.**	25	-	-24	-13	8	-	-12
12. Jul	**wide cl.**	47	24	-	11	32	24	12
23. Jul	**shad.**	41	13	-11	-	21	13	1
13. Aug	**timb.**	15	-8	-32	-21	-	-8	-20
05. Aug	**SS_Ta**	26	-	-24	-13	8	-	-12
24. Jul	**DWD**	37	12	-12	-1	20	12	-

Results from the comparison of development rates agree widely with those found for the temperature data itself in the previous chapter. However, the calculation of development rates provides additional information. In April (grey oval), calculated development rates reached comparable levels at all spots with 5 to 10% completion at the end of the month but for the wide clearing, which exceeded almost 20%, and the soil which was too cold for development.

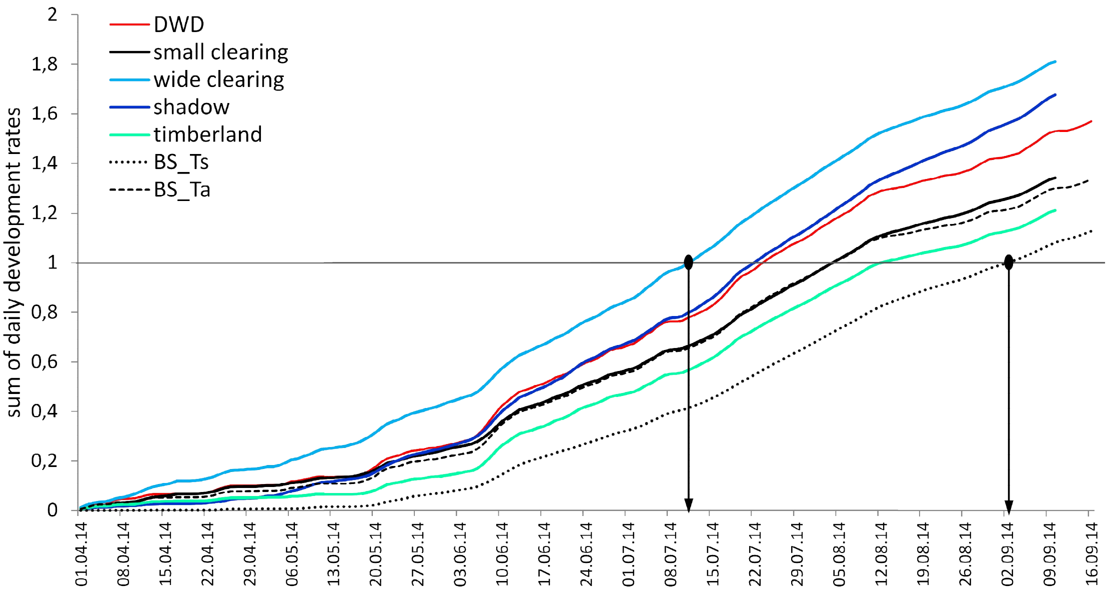


Figure 4.28: Egg development calculated by different temperature data measured at Gosheim (GH). The horizontal line depicts the state when development is completed (DdR_{sum} = 1), while the black arrows mark the first and last date of development completion, calculated from data of different spots.

From May to the end of investigation, development conditions in **timberland** stayed lower than at all other litter spots and ended one month (32 days) delayed compared to the warmest spot (wide clearing). The **hot period** in June (Figure 4.25, middle grey bar) is also visible here as a steep increase of development (grey bar). As expected, eggs had completed their development at the sun radiation favored spot **wide clearing** at first, in early July, and reached up to 180% of development in total. Interestingly, this value of 180% is comparable with conditions found at the north-faced timberland in Wüstenrot (WR) at about 500 m a.s.l. (see Figure 4.17) in 2014.

Of course, data from the nearby **official weather station** (DWD) do not represent the wide variance of development conditions found at Gosheim. Nevertheless, the development course calculated with these data represented more or less the averaged conditions found at the four litter spots.

4.5.3 LITTER LAYER AND HUMUS TYPE

Researchers widely agree on the importance of the litter and upper soil layer for living processes and survival of *I.ricinus* ticks (Kahl 1989, Sonenshine & Roe 2014). They spent most of their lifetime within this microhabitat, search shelter when weather conditions are rough and regain water losses suffered from active questing. Surprisingly, little has been done to explore this habitat and its influences on ticks in detail up to the present. Thus, the following examination aims at providing new perceptions concerning the ticks' major microhabitat.

The statement that ticks are able to regain water losses in the litter and upper soil layer (Schwarz et al. 2009, Li et al. 2012) implicates the assumption that the litter provides suitable humidity conditions (> ~85% RH). Looking at experimental results by Putuhena and Cordery (1996), the capacity to store water decreases with shrinking litter mass. Combining the latter information, one should expect that the ability to regain water losses for ticks should be less in habitats of a sparse litter layer compared to habitats with a rich layer. Concluding, ticks are expected to be much more sensitive to drought and show a more highly mortality and smaller abundance, respectively, in habitats with a sparse ground coverage, especially in seasons with long dry periods.

As a consequence of this and to simplify the complex humus type characterization, I distinguished two groups of humus with tick-relevant characteristics for further analysis:

I. **Constant types** like moder and raw humus, which keep a certain litter mass throughout the year and are built up by two or more layers

II. **Temporarily types** like mull, whose litter is often built up only by leaves of the last year can be depleted at the end of summer due to fast decomposition

Figure 4.29 illustrates the humus situation of these groups in winter (suppressed decomposition), spring and summer (decomposition) with the temporarily types denoted as "mull" and the constant types denoted as "moder". As visualized, mull-type humus is characterized by a fast decomposition that can lead to a consumption of almost the whole litter body until the next leaf litter falls in autumn.

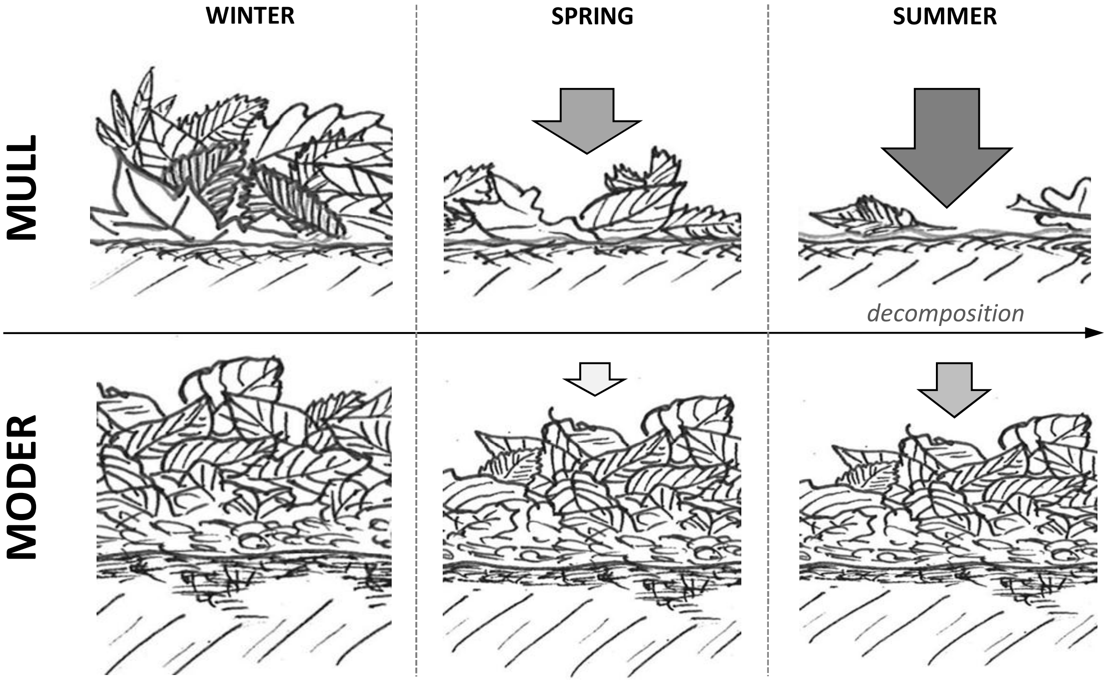


Figure 4.29: Decrease of the mull (top) and moder/raw (bottom) humus mass over the tick season due to decomposition processes (author's own drawing).

The humus types investigated during the study were classified as:

AW: **mull** (mainly build up by fresh litter)
HW: **moder** (medium)
MB: **moder to raw humus** (thick)
SW: **raw humus** (thin)
DS: **mull** (with a certain amount of only partly decayed litter)

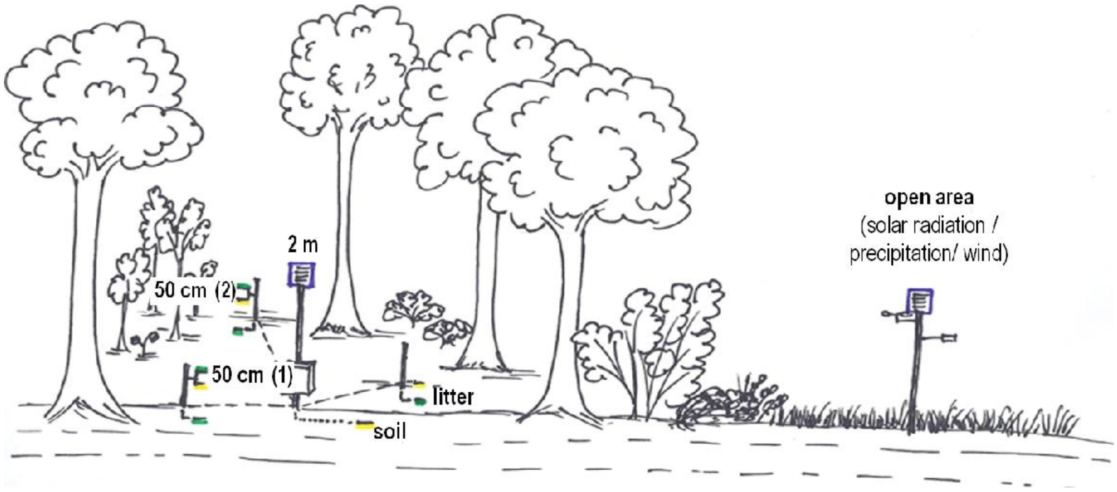


Figure 4.30: Measurement setting at Intensive Sites. Labels indicate different domains. Yellow = moisture sensors, green = temperature sensor (author's own diagram).

Auwald (AW) is the most representative study site for the mull type, Michaelsberg (MB) the one for thick raw humus like moder concerning the microclimatic measurements in the litter layer. It has to be noted that interpretations are limited because litter data were measured only at one spot within a highly variable microhabitat.

The focus of the analysis is on two humus types (constant and temporarily), their characteristics of temperature and moisture conditions and a deeper understanding of relevant processes. Data recorded within Intensives Sites form the basis of the analysis; the measurement setting is depicted inFigure 4.30.

To investigate the role of the humus layer as a transition zone between the underlying soil and the overlying subcanopy air masses, the daily courses of temperature and relative humidity were examined on a monthly basis at different domains inside and outside the forest. To gain comparable results, analyses are again based on data of the 2014 season, as in the previous chapter "effects of stand density".

4.5.3.1 EFFECTS OF HUMUS TYPE ON TICK DENSITIES

Since *I. ricinus* is highly sensitive to desiccation, a positive influence of humus types with a high capacity to retain moisture on tick survival and thus tick densities is expected. Indeed, evidence of a relationship between more highly tick densities and thicker litter layer was found by surveying tick densities in different forest types, e.g., in the Netherlands (Gassner et al. 2011), England (Medlock et al. 2012) and Northern Spain (Estrada-Peña 2001). In turn, insufficient shelter of a sparse litter is expected to lead to a more highly mortality and thus lower tick densities, especially in times of drought.

As seen in Chapter 4.1.2, tick activity was very high in spring 2014 but fell down and never reached more highly levels over the rest of year. In addition, tick densities were much lower in 2014 compared to 2013. It is possible that these findings are explained by the warm and too dry conditions in spring 2014, leading to dehydration within the ticks' environment (and thus a more highly mortality rate of ticks). Since mull humus seems more vulnerable to desiccation, especially with shrinking litter mass, this subchapter focuses on tick densities found at study sites with mull type humus to examine relationships.

A very rare litter mass due to a fast turnover of litter (illustrated in Figure 4.29, MULL + SUMMER) was observed at the study site Kirchheim unter Teck (KT) and, to a lesser extent, at the riparian forests Auwald (AW), Altenheim (AL) and Neckaraue (NA) in autumn 2014.

Table 4.12 gives an overview on the tick and vegetation situation of these study sites where fast decomposing mull humus was observed.

Table 4.12: Nymphal densities (ND), percent changes of nymphs from 2013 to 2014 and the vegetation parameters litter depth (N = low), litter species (litter spec: B = beech, E = oak, L =other leaf types) and incidence of the herbage (N = low, M = medium) for study sites with a mull type humus.

	ND 2013	ND 2014	changes	RHsum13	litter depth	litter spec.	herb inc.
KT	131	8	- 0,94%	89 %	N	BEL	N
AW	226	163	- 0,27%	89 %	N	L	M
AL	263	374	+ 0,42%	84 %	N	EL	M
NA	138	73	- 0,47%	88 %	N	L	M

Despite the overall decrease of tick densities, the total nymph density in 2014 was outstandingly low at KT (8 nymphs), since more highly numbers are to be expected looking at the suitable site conditions (broad-leaved forest, medium altitude, annual mean RH > 90%). This study site was the only one that combines a low litter depth (especially at the end of summer) with a sparse herbal coverage. In contrast, the other study sites had a medium herbal coverage at the end of summer. However, two of these three sites are characterized by a remarkable reduction of nymphal densities from 2013 to 2014. This leads to the assumption that the reduced sheltering functions of the mull litter had a negative effect on the survival chances of ticks in 2014.

The following chapters thus aim at analyzing conditions provided by the litter layer and at describing connected processes and pattern.

4.5.3.2 CONDITIONS IN SPRING OR UNDER DEFOLIATED AND THIN FOREST STANDS

Processes have been found to differ mainly according to the three characteristics: humus type, forest type and season of the year. This part describes conditions occuring when large parts of solar radiation directly reaches the forest floor, i.e., in spring when trees are defoliated or at thin forest stands all over the year.

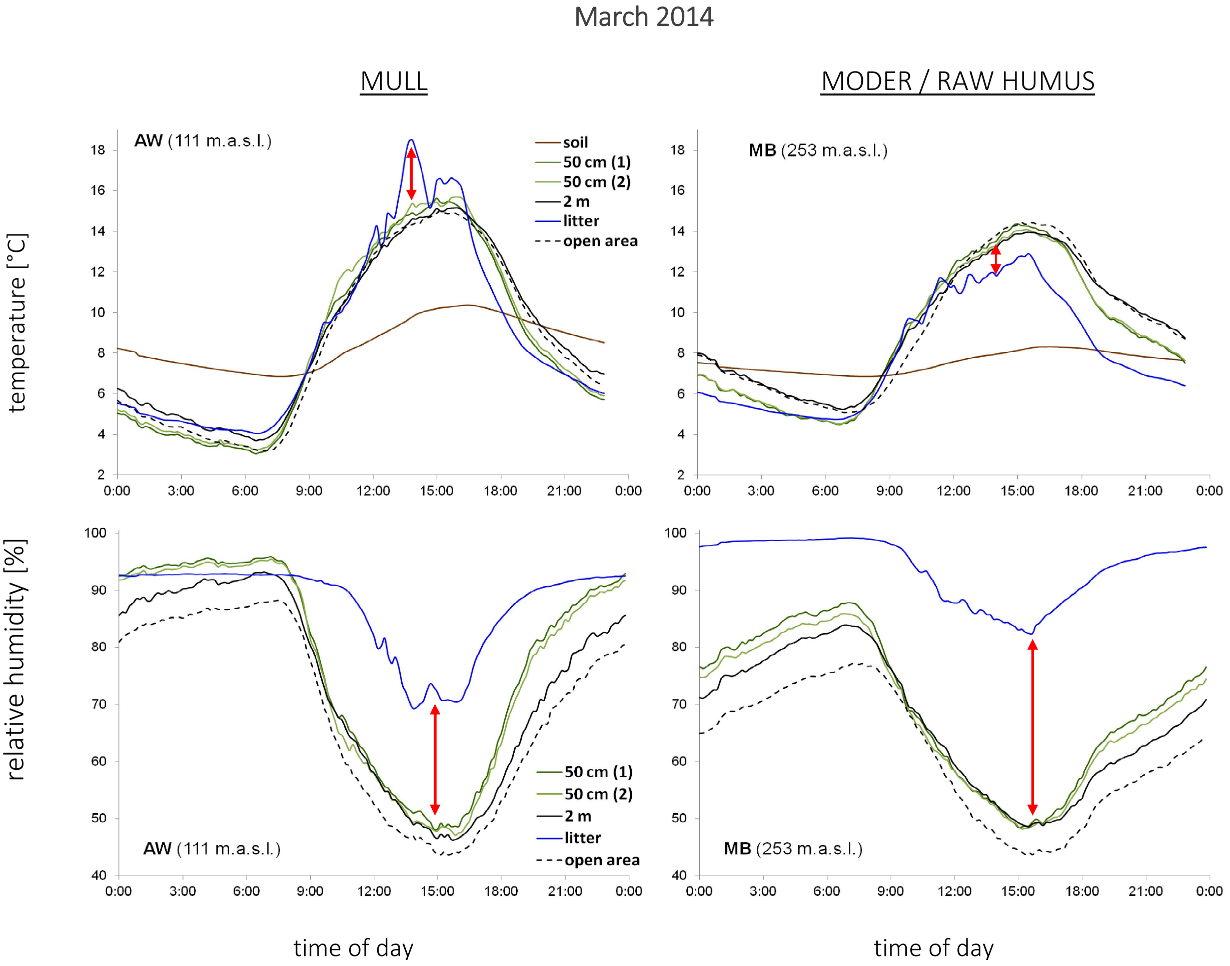


Figure 4.31: Daily course of temperature (top) and relative humidity (bottom) at different spots in Auwald (AW) and Michaelsberg (MB). The 10 min values are calculated as monthly averages of data in March 2014. Litter temperature courses (top, blue) differ noticeable at daytime between the two humus types mull and moder / raw humus. Relative humidity in the litter (bottom, blue) stayed higher under moder/raw humus (MB) compared to mull (AW), even if the atmospheric conditions were in total drier at MB

The diurnal temperature course in the mull (left) versus the moder humus (right) had their highest deviation in spring. The results are illustrated by the example of March 2014 in Figure 4.31. **Air temperature** courses (50 cm and 2 m) in the forest and at the open area showed a similar diurnal trend in March 2014. The **soil temperatures** under mull-type humus show a higher increase at daytime compared to the soil under moder humus. The **litter temperature** of the mull-type humus rises above the air temperature (50 cm and 2 m) during the day, while the litter temperature of the moder and raw humus (on the example of MB) stays lower. In addition, the litter temperature shows several peaks, while air temperatures have a smooth diurnal course.

The daily course of relative humidity slightly shows an increasing trend from outside to inside the forest (open area versus 2 m) and, in addition, from upper air layers to lower air layers (2 m versus 50 cm). At night, the relative humidity increased much faster and to a higher extent in Auwald (AW) compared to Michaelsberg (MB).

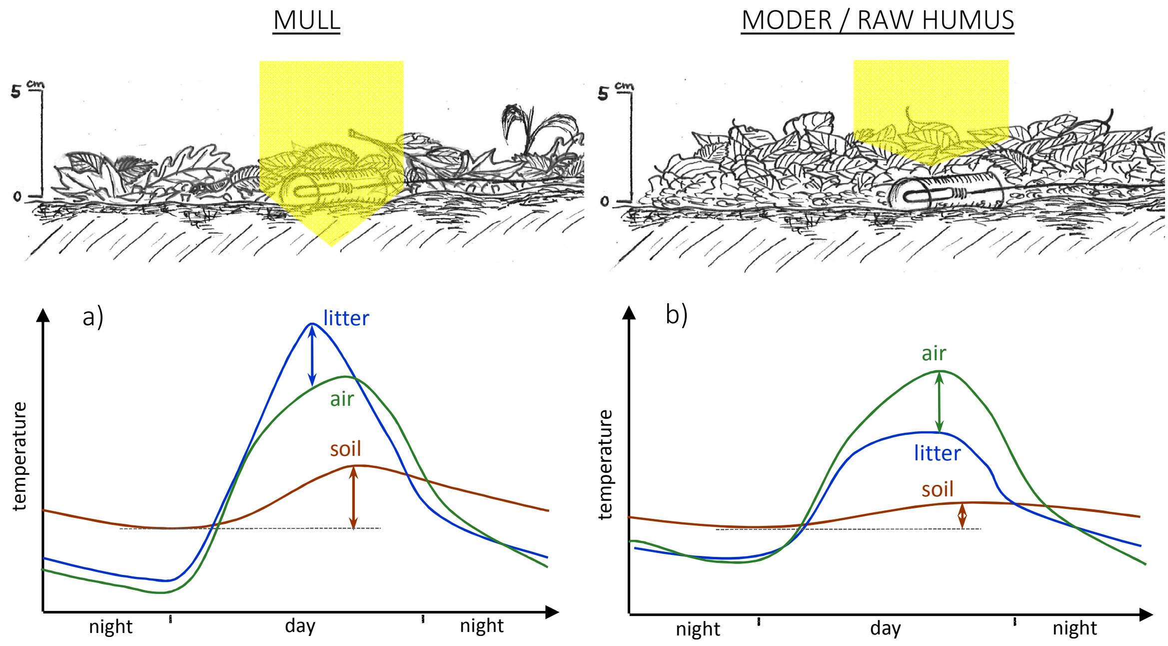


Figure 4.32: Qualitative model of the daily course of temperature conditions in the litter layer, the air layer above and the soil beneath for a) mull type humus and b) moder type humus (bottom). The drawing on the top illustrates the effect that lead to the different daily courses: The incoming solar radiation is better buffered by moder compared to mull. This situation refers to conditions when solar radiation is absorbed by the litter layer, e.g. in thin forest stands, under defoliated canopies and, to a lesser extent, due to temporarily sun spots in dense stands.

Despite its more humid air conditions, AW had **drier conditions within the mull** type litter compared to the moder litter in HW. At daytime, the relative humidity dropped down to 70% (monthly average value!) in AW but stayed within the range of the ticks' equilibrium humidity from 100 % to about 85% in HW. To give a seasonal context, March 2014 was too warm (about

3.5°C) and much too dry with only one third of the precipitation amount compared to the long-term average (DWD, press release; DWD 2014).

A qualitative model of the temperature related processes is illustrated in Figure 4.32.

4.5.3.3 CONDITIONS IN SUMMER OR UNDER FOLIATED AND DENSE FOREST STANDS

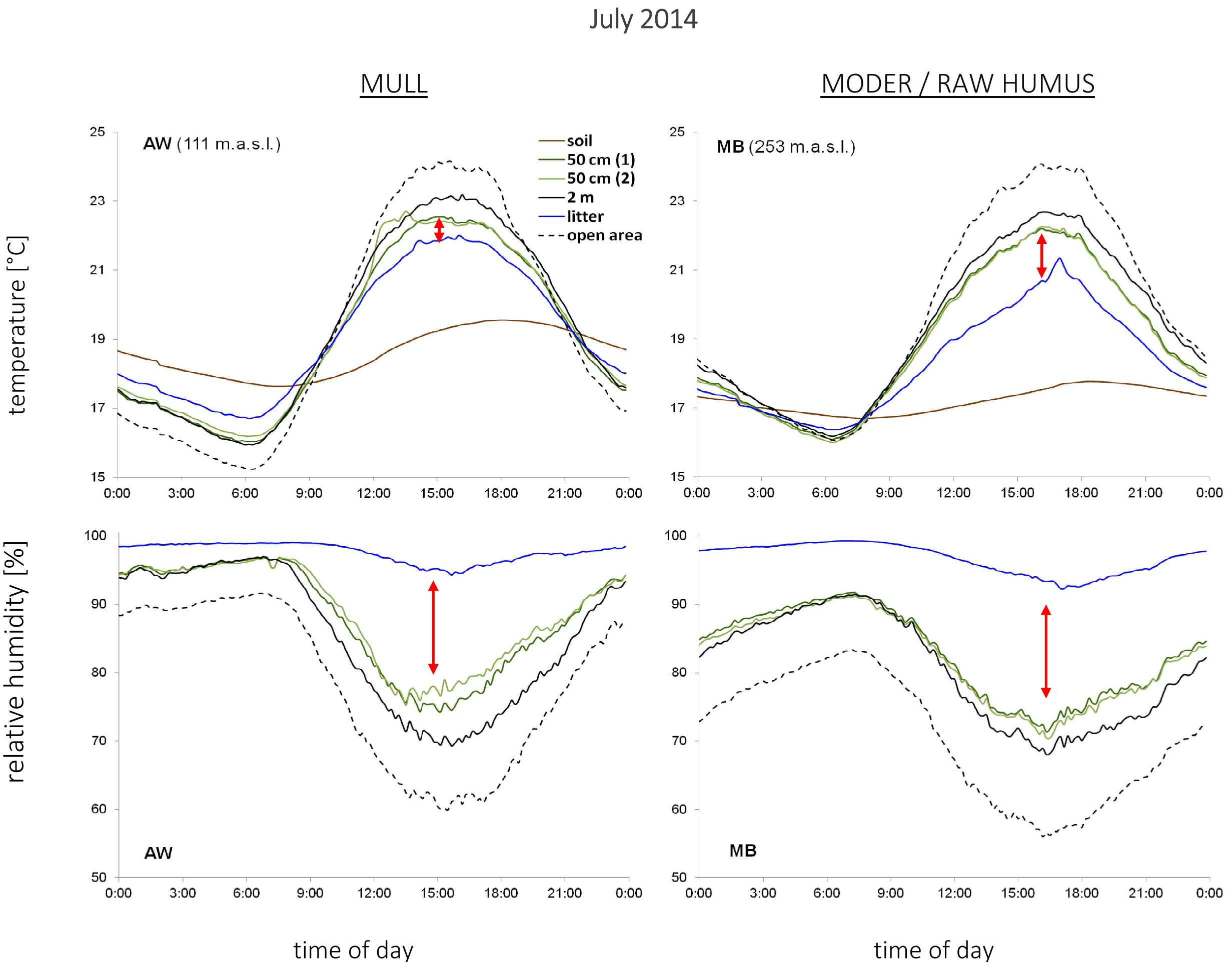


Figure 4.33: Daily course of temperature (top) and relative humidity (bottom) at different spots in AW and MB in July 2014. Compared to conditions in March, temperature and relative humidity were more similar underneath the two humus types (litter, blue) but more different at atmospheric spots, especially from inside (2 m, black solid) to the open area (dashed line) and from top to bottom (black > green > blue).

Figure 4.33 demonstrate the effect of foliation on temperature and relative humidity courses under broad-leaved trees using the example of July 2014.

In contrast to those in springtime, **air temperature** courses inside and outside the forest show different amplitudes over the day in summer. The official weather data are not included, since the timely resolution of 1h did not fit the high resolution of 10 min, but it is very likely that the daily temperature amplitude is even higher than the one of the sensor located outside but nearby the forest (open area). The **soil temperatures** show similar pattern, like those in springtime, with a higher temperature increase under mull compared to moder. The **litter temperature** courses stayed below those of the air temperatures at daytime in both humus types, but showed a lesser warming under moder compared to mull. A temporal restricted warming due to sunspots can be seen at MB at about 15:00 (UTC).

According to the temperature courses, the **relative humidity** showed a much higher variety between the different spots in the air (50 cm, 2m) compared to springtime. In addition, the increasing trend from outside to inside and from top to bottom comes out much clearer. Similar to springtime, the air humidity conditions were wetter in total in AW compared to HW. Humidity in the litter layer stayed high (> 90 %) over the entire day under moder as well as mull at both study sites. To give a seasonal context, July 2014 was too warm (about 2.3 °C) and much too wet, especially in the country Baden-Württemberg achieving an all-time record of 200 l/m^2 (DWD, press release; for example (DWD 2014)).

On the example of the study site Michaelsberg, the daily courses of temperature and humidity are illustrated for several month in 2014 to illustrate trends in the course of a year (see Appendix (C6)).

4.5.3.4 THE EFFECT OF SOLAR RADIATION IN SPRING

On the example of conditions recorded at the still defoliated riparian forest Auwald (AW) in early spring 2014, some effects of directly incoming solar radiation on humidity in the litter layer are demonstrated in Figure 4.34. The focus is on the difference between air conditions (50 cm and 2 m) compared to the litter layer where the solar radiation is absorbed. Microclimatic parameters used for this comparison are: measured temperature and relative humidity as well as the calculated absolute humidity. A period is presented with a relative humidity of 100 % in the litter layer over the entire time (middle Figure).

Differences between air and litter conditions were small at days with a low solar radiation, e.g. at 26th February. At sunny days, e.g. at 9th March, the two domains showed strong diurnal differences in moisture conditions. Along with the absorption of solar radiation, the temperatures increased from about 3° at night to almost 20°C at day on both domains (bottom). Consequently, the relative humidity (Figure 4.34, middle) decreased proportional and the absolute humidity (Figure 4.34, top) stayed at a constant level in the atmosphere (50 cm / 2 m). In contrast, the relative humidity under leaf litter stayed at 100 % and the absolute humidity increased from about 8 to 15 g/m³. At night, the absolute humidity declines strongly in the humus but stays higher than in the athmosphere.

Interestingly, the observed rising of litter temperature above the atmospheric temperature at daytime in March 2014 (see Figure 4.31) occurred seldom within this period. The litter temperatures exceeded those in the air only at four days (28.2 to 2.3. and at 10.3.) even if the amount of solar radiation reached high levels at day, e.g., from 4.3. until 9.3.

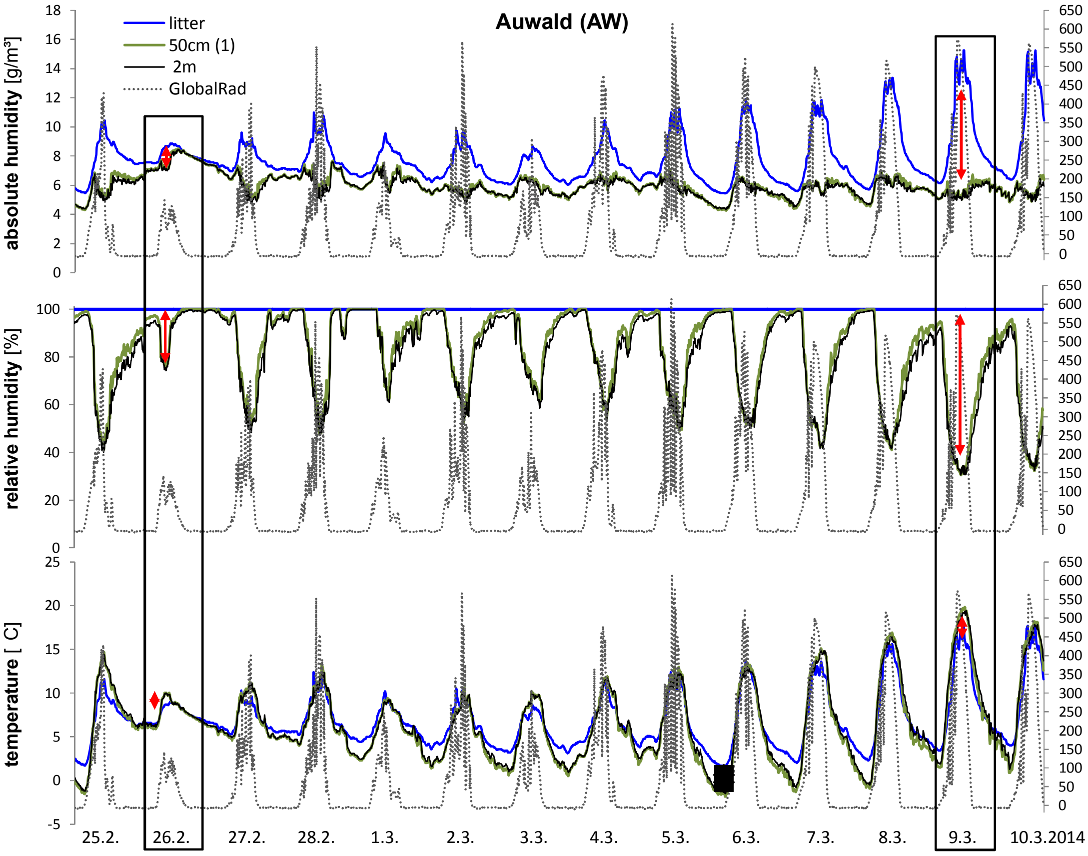


Figure 4.34: The effect of global radiation on conditions in the litter layer compared to atmosphere (50 cm / 2 m) in respect to absolute (top) and relative humidity (middle) as well as temperature (bottom) in early spring 2013 at AW. Arrows depict the extent of radiation induced, diurnal differences between litter and atmosphere. Differences rose along with the amount of incoming solar radiation. The relative humidity stayed at 100 % in the litter (blue). As a result, absolute humidity increased along with temperature at day.

4.5.3.5 THE EFFECT OF PRECIPITATION ON MOISTURE AVAILABILITY

As seen in the last chapter, a sufficient amount of liquid water stored within the humus balances interior humidity conditions so that they remain suitable for resting ticks. This stored amount of liquid water is expected to depend on 1) precipitation input and 2) storage capacity of the litter body following results from Sato et al. (2004). Thus, the following data analysis focuses on this matter. As a first result, the effect of rainfall on soil moisture is illustrated in Figure 4.35 on the example of Gosheim over the season 2014. More analysis according to this example are presented in Chapter 4.5.2 (effect of stand density). Since spring was comparably dry and warm in 2014, soil moisture was down at about 15% [m ³ water/ m³ soil]. After rehydration through rainfalls in late April, moisture was reduced again frequently at dry and especially hot weather phases, e.g., by a heatwave from about 30% to 20% within two weeks. Heavy rainfall in July rehydrated the soil again up to 30% (= maximum value of this soil).

Very interesting is the effect of the warm and dry phase in spring 2014 on the humidity in the litter layer, especially since tick densities stayed low over the rest of the season after showing an intensive activity in spring. The soil moisture in Gosheim was down at 15%, how did the moisture in the litter layer of other study sites react on this situation?

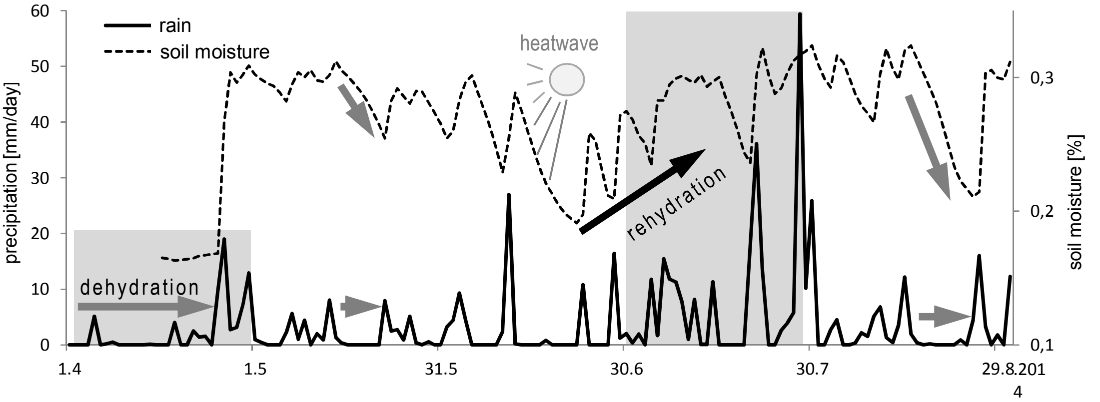


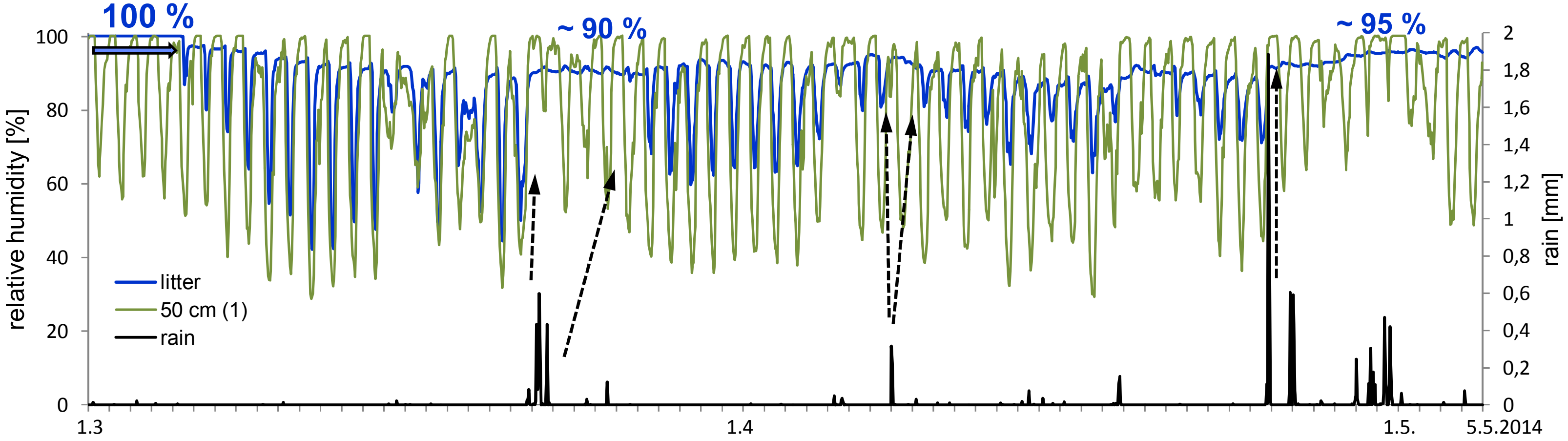
Figure 4.35: Effect of rainfall on soil moisture content at the example of Gosheim in the tick season 2014.

Figure 4.36 gives an answer to this question according to two different humus types. The study sites AW (mull) and MB (moder to raw humus) are in close proximity (about 10 km) and thus comparable to weather occurrences. Both sites are characterized by broad-leaved forest, thus trees were still defoliated at beginning and foliated successively from March to May. Therefore, rainfall reached the litter layer directly at both sites, especially in March.

Both study sites had constantly conditions of 100% relative humidity (RH) in the litter layer at the beginning, which lasted at MB five days (to 10th March) longer than at AW. After that, RH dropped down at day at both study sites, but never reached 100% again in AW in contrast to MB.

Rainfall in the second half of March rehydrated the moder in MB up to 100% constantly for about 4 days and this 100 % level occurred frequent at night over the entire period. Only when the atmospheric RH at 50 cm stayed low over days, e.g. on start of April, conditions in the litter were dehydrated below 100%. In contrast, the mull (AW) never reached this 100 % level again but stayed at 90 to 95 % at maximum, even if conditions at 50 cm often reached 100% RH at night.

MULL (AW)



MODER / RAW HUMUS (MB)

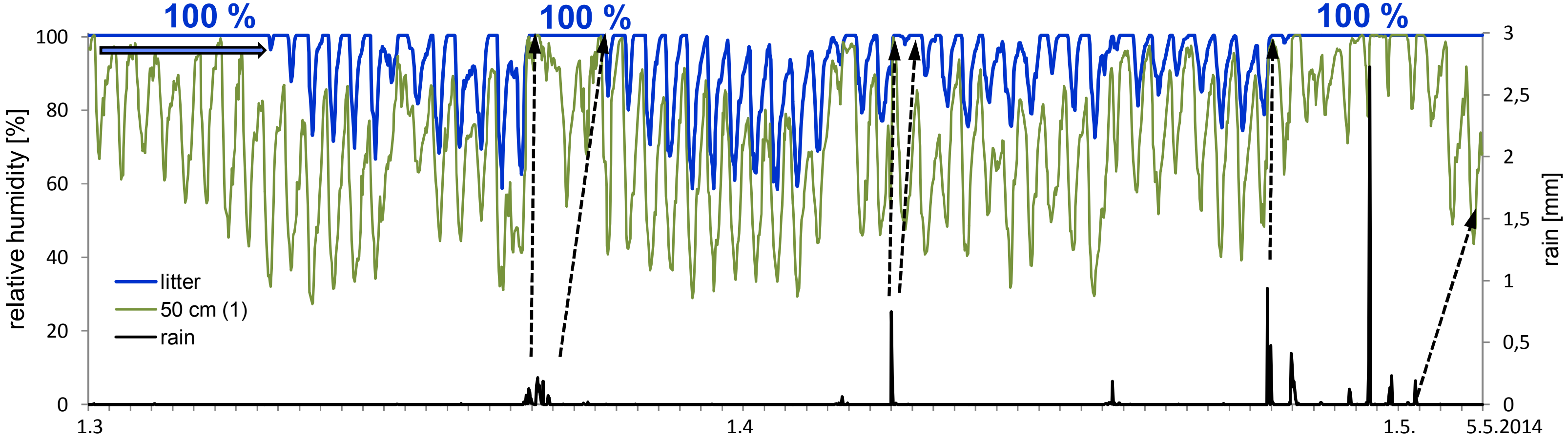


Figure 4.36: Relative humidity in the litter layer (blue labels) under mull (top) and moder / raw humus (bottom) and in the nearby herbage (50 cm) during the warm and dry spring season 2014. Dashed arrows illustrate the effect of rainfall on RH. The moisture availability for ticks was much better under moder compared to mull humus.

4.5.4 DISCUSSION AND CONCLUSIONS

PRELIMINARY STATISTICS

The test yielded no results that indicate a constant influence of vegetation on tick densities. Only in 2013, some vegetation parameters affected nymphal densities significantly. If these results are not only an artifact of the (statistically) low number of tested sites, they could indicate a yearly changing suitability of specific habitat characteristics for *I. ricinus*, probably due to weather occurrences. For example, the **litter depth** could play a major role in years with hot and dry conditions (especially in summer), when forests with high litter depth offer ticks moist shelter suitable to survive in contrast to those with sparse litter (according to results of Schwarz et al. 2009), since the litter depth is related to the water storage capacity of the litter (Putuhena & Cordery 1996, Park et al. 1998). The **forest type** could be important in years with a cold spring, such as 2013. Coniferous forests, constantly shading the forest ground, could delay the activity start and development of ticks additionally and thus decline the chance for a successful surviving to the next life stage (explained in Chapter "discussion temperature chapter").

However, there is another way to interpret these results. The test checked if groups divided after vegetation characteristics have different medians according to nymphal densities. As seen later, the significant vegetation parameters are not uniform distributed over the altitude range but mostly concentrate at low or high altitudes, according to ecological terms. Thus, the strong influence of the temperature or rather altitude of the study sites on tick densities could scatter the interpretation of results, for example those related to forest type (see results and related discussion in Chapter 4.3.1.2 and 4.3.4). Since coniferous forests (and, accordingly, the litter species "needle") are restricted to higher altitudes, their tick densities are naturally low compared to other forest types and litter species.

This forest type distribution reflects the current forest type distribution over Baden-Württemberg where, e.g., the main part of the coniferous forest area is located at the mountain *Schwarzwald*, as shown in Figure 3.1. Interestingly, study sites with low litter depths concentrate at low altitudes, while medium to high litter depth are more or less equally distributed. One explanation for this is a higher decomposition rate at lower altitudes due to warmer temperature conditions (if moisture conditions are sufficient). *I intended to show the reader how difficult it is to identify relevant factors and how these interpretations are broadly influenced in* *time and space. The more important are results gained by process-oriented analysis discussed in the following.*

EFFECTS OF FOREST STAND DENSITY

HOW THE LOGGER CONFIGURATION INFLUENCES RESULTING TEMPERATURE DATA

First, data from two logger configurations were compared. As expected, it was found that the modified loggers detected temperature extremes better than the original loggers and that differences between the two logger configurations were greater at the clearing compared to the shadow spot.

This leads to an important outcome: if we want to measure data in the field that can really represent ticks environment, for example to record conditions test-ticks in microcages are exposed to, we best place the sensor in the cage and measure directly. A compelling practice was done by Materna et al. (2008). They observed test ticks in the field, placed on the soil and covered with leaf litter, and compared them with data measured "5cm above the ground" by "sensors placed in a white radiation shield". However, even these data cover a mixture of ground-near atmospheric conditions rather than the actual conditions below the litter layer, where ticks are placed, and thus hinder an exact evaluation of the influence of the microclimate on tick mortality, especially for the relative humidity factor (compare Chapter 4.4.2).

Following examinations were done with data from modified loggers to image the broad variety of actual occurring temperature conditions ticks are exposed to rather than averaged conditions.

TEMPERATURE CONDITIONS

Data showed a high variety of ground-level temperature conditions changing diurnally and seasonally within the forest area. Data from the official weather station imaged more or less an average of conditions found at the litter spots - looking at the development progress and averaged daily temperature courses. In this case, these data were appropriate to express medium temperature conditions of the total forest area. It therefore seems that the free accessible, official weather data give a good estimation of temperature conditions for a first start, for example to select research locations. However, temperatures in well shaded areas such as timberland (or northern slopes) would be strongly overestimated, while ground temperatures in open areas (or southern slopes) would be underestimated using official weather data instead. *Thus, the exclusive use of official temperature data has to be considered carefully depending on the research question, in order to get reliable results.*

IXODES RICINUS ABUNDANCE AND DEVELOPMENT

The development progress over the season varied considerably between the different litter spots, leading to a time-difference for egg development completion of about one month between the warmest and coldest spot.

Since egg development could actually be completed all over the place until mid-August 2014 the latest, the results fortify the hypothesis that this study site of high altitude, Gosheim, provides suitable temperature conditions for egg development and, in addition, a stable tick population in general. However, even if the development duration is short enough to be completed in a season, only a small percentage of eggs actually hatches atsuch high altitudes (Materna et al. 2008) and the time span for active host questing before the winter is shortened, leading to a higher inter-stidial tick mortality (Randolph 2004) and thus to comparably low tick abundances (Medlock et al. 2013).

In addition, the initial hypothesis that stand density affects ticks living conditions by creating a mosaic of temperature pattern on the ground could be verified by this additional research approach. The area of dense timberland provided the coldest and the wide clearance the warmest conditions for ticks in this area, according to the amount of directly incoming solar radiation warming the ground surface (Geiger et al. 1995). Accordingly, tick densities were higher at warmer parts than at the cold timberland, even if not at a statistically significant level. Since the temperature budget is a critical factor at this high altitudes, it seems very likely that tick densities are modified by the factor stand density as a result of the different warmth availability at the ground. Concluding, tick densities should be higher in medium to thin forest stands where the fragmentary canopy leads to a high amount of temporarily warmed patches on the ground compared to stands with a fully covering canopy. According to results of Chapter 4.3, these effects likely occur at altitudes above 500 m a.s.l. and increase along with altitude.

These results also explains why *I. ricinus* was found less common in pure and tight coniferous stands of higher altitudes (AH and VS with 3 to 37 nymphs/year), compared to tight mixed or broad-leaved stands (DS, SW with 47 to 166 nymphs/year) with a comparable altitude of 610 to 770 m a.s.l.. The evergreen coniferous forest shades the ground throughout the year and thus constantly reduces its warming and the development speed of inhabiting ticks. In contrast, a high amount of incoming solar radiation reaches the ground in a broad-leaved forest in springtime. The temporarily warming in spring support a faster tick development before the foliation of trees is completed compared to constantly shaded habitats.

Cadenas et al. (2007) had comparable results comparing tick densities on a north-facing (cooler conditions) and a south-facing (warmer conditions) slope at altitudes from 620 m to 1000 m a.s.l. (= area of restricted temperature budget, see Chapter 4.3) – higher tick densities occurred at the temperature favoured site.

Concluding, the latter results all fortify the limiting power of temperature for I. ricinus ticks at higher altitudes and, resulting, a strong impact of ecological factors influencing the ground temperature such as stand density, forest type and sun exposure of the habitat. Future tick research projects should therefore consider such impact factors in their sampling approach and interpretation of the data, especially at altitudes where temperature in general acts limiting for I.ricinus living processes.

TICK SAMPLING DATA

To improve the reliability of the tick data and to reduce the influence of weather occurrences at the results using the flagging method for tick sampling should be avoided in future, especially for short-time projects and when comparing nearby tick populations. One problem with sampled ticks (except for larvae) at different parts of one connected forest area is that they do not origin from the place captured, since they were previously passively transported to their new spot. So there presence partly reflects suitable conditions at their previous (micro)habitat.

To get more reliable results in future a quasi-natural approach is needed, e.g., by installing microcages including a known quantity of ticks (as described in Materna et al. 2008) to evaluate the actual influence of stand density and, accordingly, small-scaled microclimatic differences on tick mortality.

LITTER LAYER AND HUMUS TYPE

EFFECT OF HUMUS TYPE ON TICK DENSITIES

This specific examination (Chapter 4.5.3.1) was done under the assumption that a sparse litter cover negatively influences ticks water supply and life span. Indeed, a strong decrease of tick densities was detected at three of the four study sites with a sparse litter layer from 2013 to 2014 and, in addition, two of these sites (KT and NA) were clustered to a group of especially low tick numbers beside sufficient warm conditions (see Figure 4.13). Especially the study site Kirchheim Teck (KT) showed extreme low tick densities and it was the only site that combined a sparse leaf litter with a sparse herbal layer and high clay content of the soil (likely hard to penetrate for ticks). The other three sites were characterized by a medium dense herbal layer which probably partly replaced the sheltering function of the litter, leading to a lesser drop of tick

numbers. The results are in accordance with results from Schwarz et al. (2009) who found comparably low tick abundances at forest areas with sparse shrub, herb and litter coverage combined with a low soil moisture in five different deciduous forests of the Siebengebirge in Germany in 2003. However, they tested only the relationship between soil moisture (measured once a week) and tick densities and found a positive influence of humid soil on ticks.
Considering the new insights in the litter layer, its functions as a water reservoir, the influence of precipitation on humus and soil moisture (the soil near the surface, as measured by Schwarz et al., is especially moist after rainfall as is of course the litter layer as the covering storage medium), it seems very likely that not the soil water content (alone) is responsible for higher tick abundances but rather the thickness of the leaf litter, i.e., the humus type.

CONDITIONS IN SPRING OR UNDER DEFOLIATED AND THIN FOREST STANDS

It was found that the humus type has a strong effect on microclimatic conditions within the humus and nearby soil layer in spring. Conditions under humus showed in general a lesser diurnal microclimatic amplitude compared to those measured in the air, except for the temperature under mull humus where peaks of local warming were observed at day. In addition, the relative humidity stayed considerably higher within the litter layer at day compared to the air, but dropped down to a higher extent within the mull humus compared to the moder / raw humus.

These findings are not surprising, since the buffering capacity of humus declines with shrinking litter masses (Putuhena & Cordery 1996, Park et al. 1998). In March, trees were still defoliated so solar radiation was absorbed mainly at the forest floor and the litter layer, respectively. The litter sensor measured directly on the ground and was covered by litter. The mass and depth of the insulating layer was higher at the moder humus compared to the mull humus. Thereby, incoming solar radiation was effectively absorbed within the upper litter layer of the moder humus and temperature at the litter basis und the underlying soil stayed cooler. In contrast, the thin litter layer of the mull was not able to insulate underlying parts, so that the litter as well as the soil temperature noticeably rose at day.

The illustrated qualitive models (Figure 4.32) are not only based on the results of Auwald (AW) and Michaelsberg (MB), but include the site Drackenstein (DS) with a mull type humus and Hardtwald (HW) with a moder type humus which show very similar trends according to their litter type (see Appendix (C7)). Based on these findings it can be expected that the daily courses of temperature and moisture in spring generally have similar trends according to the litter type, under comparable weather situations. An aside: The latter considerations do not include parameters that could have an effect on the soil warming rate like soil type and moisture content.

Looking at the effect of these findings on ticks living processes leads to the assumption that the reduced temperature (Figure 4.31 top) under moder / raw humus in spring delays the activity and development start for overwintering ticks at those forest habitats compared to habitats with a mull type humus, where a faster warming of the humus body supports an earlier onset of ticks living processes (compare Chapter 4.3 and Dautel 2010). This could also partly explain the high variety of activity pattern found even at nearby study sites (described in Chapter 4.1.2.2). However, the monthly resolution of the tick data does not allow further investigations to that matter. The hypothesis could be verified by quasi-natural field plot experiments with modified litter masses and frequent recordings of tick activity, following the approach from Dautel et al. (2008).

CONDITIONS IN SUMMER OR UNDER FOLIATED AND DENSE FOREST STANDS

When trees are fully foliated, the main part of incoming solar radiation will be absorbed within the canopy of a forest. This applies to broad-leaved forests in summer and early autumn, as well as for evergreen coniferous forests for the entire year. The coniferous trees that are common at the study sites (*Picea abies, Abies alba, Pinus sylvestris*) are permanently foliated. Changes of radiation pattern within the stand are therefore restricted to changes of the zenith angle of the sun in the course of the year.

It was found that the fully foliated canopy creates an inner stand climate that is buffered to a high extent going from outside to inside the stand and from the air to the forest floor in accordance with executions from Otto (1994) and Geiger et al. (1995) (see also results in Chapter 4.4.2). The most buffered zone is therefore the litter layer, which provided constantly excellent conditions for ticks to regain water losses or to rest with a stable physiological water balance in July 2014. The high litter moisture, which was comparably under mull and under moder / raw humus, was supported in this case by the high amount of precipitation.

In dry and hot summer seasons, however, the water storage capacity of the litter layer is expected be much more crucial for the survival of ticks and thus the humus type becomes a relevant factor for the abundance of tick populations in a given habitat.

In addition, the results indicate that official weather data are especially unsuitable to examine relationships between ticks and climatic variables in summertime, because of the strong buffering effect of the canopy and subcanopy layers on microclimatic conditions.

THE EFFECT OF SOLAR RADIATION IN SPRING

Again, results indicate an unique character of microclimatic conditions which are composed by the humus body. Under the leaf litter of the riparian forest Auwald in March 2014 an increasing **absolute humidity** was recorded at day and, resulting, stable relative humidity conditions, which differed strongly from values measured in the subcanopy air masses. These findings slightly disagree with a conclusion from Aussenac (1999), which stated that the absolute humidity within the forest is comparable to the outside and differences between relative humidities are related mainly to cooler temperatures in the forest. Looking at our results, these conclusion has to be restricted to subcanopy air masses and is not true for the leaf litter layer, especially at day time.

The diurnal higher amount of absolute (and thus relative) humidity in the humus is most likely an effect of **evaporation** processes due to the absorption of solar radiation that turn stored water (e.g. covering the leaf surfaces) into water vapor. Simultaneously, the temperature in the humus is stabilized against intensive warming due to the evaporative cooling. Sufficient liquid moisture stored within the humus layer thus reduces its heating at day, even if high amounts of solar radiation are absorbed. Large parts of the emerging water vapor remain in the humus body, depending on its capacity to withhold it against the dryer atmosphere, according to descriptions of Geiger et al. (2009) and Park et al. (1998). Along with falling air temperatures at night, gaseous water re-condenses in the litter body, the absolute humidity declines and the resulting condensation heat is stored within the litter body balancing the temperature at night.

As a result of the latter processes, the humus' temperature and especially humidity conditions were well-balanced even in spring, when the protective function of the canopy is not fully developed.

In addition, results indicate that the relative humidity resting ticks (located in the litter and upper soil layer) experience will remain at high levels even if the temperature increases - as long as a sufficient amount of liquid water is stored within the humus.

THE EFFECT OF PRECIPITATION ON MOISTURE AVAILABILITY

The field measurement results accord with experimental results from Sato et al. (2004) who found that a litters storage capacity increases along with the received precipitation amount. After rainfall, the relative humidity increased remarkably at both evaluated humus types and remained at a constant high level over days. In addition, our results indicate that the duration and extent of the increased relative humidity depends mainly on the humus type, i.e., the litter thickness. The relative humidity reached higher levels and stayed constantly moist under the thicker moder / raw humus compared to the thinner mull humus. These findings agree with experimental results from Putuhena & Cordery (1996) who found that the litters storage capacity increases with litter thickness and mass and with results from and Park et al. (1998) who showed that the duration until the stored water is completely evaporated from the litter increases with increasing litter depth. A water saturated litter layer has a very small resistance to evaporation. It increases along with the amount of aerated pores within the litter layer and this amount of pores is directly associated to the litters thickness.

The hypothesis that the two-month drought period in spring 2014 was responsible for a high tick mortality and thus caused the observed low tick densities for the rest of year is confirmed by these results. From March to May 2014, only short periods were detected were ticks were constantly able to regain water losses in the litter layer, even in the thick moder/raw type humus. Usually, relative humidity dropped down below the equilibrium threshold of ticks water balance of about 85% (Kahl 1989) at day. Under mull type humus RH increased only at about 90% in average at night, slowing the regain of water losses compared to the thicker moder/raw humus with mostly 100% RH. If ticks loose water they are forced to balance it by active water uptake, which costs energy, leads to a faster energy exhaustion and thus higher mortality rates (Randolph 2004). Thus, a frequent drop of humidity under the equilibrium threshold of ticks is expected to decline ticks remaining lifespan substantial. Since ticks need to find a host to survive, they likely increases their active questing effort – which we recognized by sampling ticks via flagging as high tick densities in spring 2014. The latter considerations agree with the explanations of the recognized expert in ticks physiology and phenology, Olaf Kahl, who stated that an increased activity of ticks in spring 2014 lead to a fast energy exhaustion and a following drop of tick numbers for the rest of season.

Interestingly, no statistically significant influence of the litter thickness on tick density was detected for 2014 (see results in Chapter 4.5.1), only in 2013. It seems that in times of too low precipitation the positive effect of the litter on ticks possibility to retain water losses is neglected for all kinds of humus, and therefore also the impact of humus related factors on tick densities. Instead, the drought period in spring 2014 had a major influence on tick behavior and survival, most likely in combination with the abundance of suitable hosts. The ticks' chance to find a suitable host and survive the drought period increases along with the abundance of hosts (Estrada-Peña & de la Fuente 2014) so I expect the factor host abundance to be very relevant under such weather conditions. Missing data on host abundances, however, limit further analysis.

To verify the actual influence of such weather occurrences on tick mortality and behavior in future activity plots following Dautel et al. (2008) or quasi-natural approaches measuring tick mortality after Materna et al. (2008) are needed to gather appropriate tick data and, of course, a suitable microclimatic measurement concept.

The following portrays an alternative weather szenario with a higher amount of precipitation in spring 2014. To the opinion of the author, a higher amount of precipitation would have reduced the microclimatic differences between both humus types comparable to those in summer 2014, because 1) the moisture would depend less on the long-term water storage capacity of the litter but more on the amount of incoming precipitation (not yet restrained by foliage) and 2) the warming would be highly influenced by material properties of the stored water and, in addition, warming would be reduced due to the cooling via evaporation. As a result, conditions would be more appropriate for tick survival in spring and lead to a higher amount of questing ticks in the rest of year. If summer is characterized by less amounts of rain, as expected in future for Baden-Württemberg as a result of climate change (Ittershagen 2015), the litters capacity to store and retain water from evaporation could be much more crucial for tick survival thus leading to higher mortality rates and lower tick densities in forest stands characterized by low litter masses. However, long drought periods could neglect the effect as seen for spring 2014.

In conclusion, the factor "humus type" should always included to describe the habitat suitability for ticks, but a detectable influence on tick densities depends also on weather occurrences which should be considered for the interpretation of the results.

METHODOLOGICAL CRITIQUE

The data measured under different humus types indicate that the buffering capacity against warming and moisture losses grow along with a growing litter mass and is therefore higher in moder/raw humus compared to mull humus. Measurements in such a diverse microenvironment are expected to be little representative. Regardless, data showed comparable trends in each group of mull type humus (AW and DS) or moder/raw humus (HW and MB), which were expected regarding to and in consensus with, respectively, results from Putuhena & Cordery (1996) and Park et al. (1998). These findings fortify the suitability of the used measurement technique to illustrate microclimatic characteristics of different humus types. However, one restriction has to be made. The sensor is placed within a tube of plastic, which is open only in direction to the ground in order to measure small-scaled averaged conditions (about 10 cm^3) rather than those at one spot. Since the plastic tube is impervious to water and the bordering litter layer is not, the tube is expected to retain more humidity and thus to overestimate relative humidity conditions, especially if water is released from the soil to the upper litter layer. How this effect could compromise results was not analyzed in detail in this thesis, since even so results fitted the expected characteristics of both humus types and thus seem reliable for descriptive analysis. Relative humidity data measured in the litter were therefore not used for tick related statistical analysis.

The results on microclimatic conditions within a forest stand indicate that tick research must include special measurements within the litter layer and groundnear athmosphere to gather appropriate microclimatic data. This concerns all research questions relating to the influence of environmental parameters on tick mortality, activity or development in forests.

The actual water availability in a ticks litter microhabitat results from a complex interplay of, e.g., water storage capacity, changing litter characteristics due to decomposition processes, amount and frequency of incoming precipitation and, of course, litter thickness. Depending on living stage, physiological state, age and fat content, respectively, ticks are more or less sensible to drought (Arthur 1962, Kahl 1989, Sonenshine & Roe 2014) and consequences of drought periods on tick behaviour and mortality are therefore variable even within one single habitat. Since dead ticks are invisible ticks for tick sampling by flagging (Sonenshine & Roe 2014), the effect of drought on tick mortality is not measurable with our approach. In fact, low tick abundances as seen in the year 2014 are a hint for a high tick mortality due to previous drought periods, but are no evidence at last. This evidence would have been given using the quasi-natural approach from Materna et al. (2008), directly measuring tick mortality in microcages.

5 CONCLUSIVE SYNTHESIS

Finally, after the intensive study of the extremely complex matter of *Ixodes ricinus* field research on the influence of environmental factors on this species, it seems necessary to first present a small but fundamental part of this complexity. This part should act as a counterbalance to the following conceptual simplifications, which are meant to make underlying processes understandable and help structure the extreme complexity in the future but, of course, concurrently suppresses ecological and methodological detail.

Field research on *Ixodes ricinus* ticks is mainly based on tick data that are sampled by flagging, where actively questing ticks are sampled from the vegetation or ground surface in a defined area. There are a lot of processes and causes that lead to the resulting data which we, a bit simplified, denote as „(questing) tick density". This amount of ticks is only the small part of actively questing ticks that we are able to catch at this very moment in a defined area. The actual amount of ticks present within this area is much larger: There is a rest of actively questing ticks, which have not been caught and a large part of ticks not actively questing. Factors that have been identified to influence the amount of actively questing ticks are the time of day (Randolph & Storey 1999), the weather (Estrada-Peña & de la Fuente 2014), the season or the length of day which initiates the ticks' diapause behavior. Not active are ticks that have found a host and are in development, ticks that restore water losses or withdraw from dryness or heat, ticks that are in diapause or dead (Dautel 2010). These non-active ticks seek shelter near the ground, e.g., within the litter layer of a forest and are therefore not flaggable. Furthermore, the amount of sampled ticks is not only based on the causes mentioned above influencing the actual numbers of questing ticks but also by causes influencing the efficiency of the sampling method: the applied sampling method (Vassallo et al. 2000), type and conditions of the flag (Jensen et al. 2000), the height and type of vegetation (Boehnke 2011) and possibly other properties of the tick collecting person. As a consequence, the more samples are combined to estimate the total population size of nymphs in forest habitats, the better the results (Tälleklint-Eisen & Lane 2000). To reduce random error effects, only data combinations of samples of an entire year (= annual tick density) are used for analysis in this thesis. On this basis, data should provide a good impression of the risk of a tick bite for a specific habitat. However, one must admit a certain difficulty if these data are used to (statistically) identify environmental factors influencing the suitability of a specific habitat.

Therefore, as a next logical step, it seems easier to examine and describe the processes and resulting (microclimatic) conditions within the ticks' microhabitat to estimate the habitats suitability.

Unfortunately, the microhabitat of ticks is the ground-level area, in particular the litter and upper soil layer for forests inhabiting ticks. This microhabitat (humus) and its changes due to decomposition processes are even more complex - in terms of the multitude of (sub-) processes influencing its actual conditions - than the just described concept of "tick density" (reviewed in Berg 2014). In addition, humus related research is primarily concerned with nutrient flows and decomposition processes but less with the microclimate within the humus layer itself, thus there are few transferable findings. The latter statement is illustrated by the number of document results of a literature search in Scopus (Elsevier): „leaf litter & microclimate": 135 , „leaf litter & climate": 871, „leaf litter & decomposition": 3657 (access on 2016-03-01).

This complexity on the one hand and little information on the other hand could be a major cause why the ticks` main microhabitat "litter layer" and its characteristics for inhabiting ticks has been widely ignored in past tick research. The complexity of the climate within forest stands and the great effort to sample those microclimatic data could be the major cause why most research approaches, trying to examine ecological relationships between ticks and their habitat, used official weather data in the past. Or, in addition, because researchers were not aware of the great deviance of conditions official weather stations describe compared to those in a tick's ground-near (forest) habitat. The latter issue should now be reduced by the findings and explanations in this thesis.

According to Berg (2014), to comprehend extreme complexities it is very helpful to identify subprocesses and, based on that, to create a conceptual model. In ecological tick science, conceptual models or superior concepts that simplify complexity are largely missing. There are, for instance, no concepts for the junction of research findings from different areas to identify key factors for the ticks' habitat suitability. Indeed, tick research tends to explain observed tick pattern on single, remarkable events (e.g., at warm winter) which should have strongly influenced tick mortality. However, since observed tick data are mostly sampled, they provide no evidence for the actual impact of a single event on tick mortality and widely ignore possible cumulative, environmental effects. On this basis, a plurality of single results was generated in the past which represent pieces of the large puzzle of environmental factors affecting *I. ricinus* populations but a master model or guideline helping to fit them together is still missing.

In this thesis, some more of this single pieces are added to the puzzle. These pieces and former findings are now combined to a more holistic overview and concepts are introduced which should help to fit these pieces together in the future.

5.1 USEFUL CONCEPTS FOR TICK ECOLOGY RESEARCH

The analysis in this thesis are based on three fundamentally different approaches: one that is based on the knowledge of the ticks' physiological needs, one that statistically identifies relevant factors and one that examines the ecology of the ticks' microhabitat. To combine and embed the findings of these approaches in a larger context suitable concepts are needed. These concepts are described in the following.

5.1.1 CONCEPT OF LIMITING FACTORS

In botany science one principal can be used to describe patterns of plant growth: the "Minimum law" formulated by Justus Liebig or, referring to van der Ploeg et al. (1999), the "Sprengel-Liebig Law of the Minimum". It states that the *factor of lowest provision* limits plant growth even if other factors are plentifully available. This concept can be adapted also to individual ticks or to tick population abundances.

Ixodes ricinus ticks are very immobile compared to other Arthropoda. When feeding on a host, ticks are passively transported to a new (micro-) habitat more or less by chance due to host specific movements. This is very comparable to plant seeds that are passively distributed via animals (e.g. burs). Their growing success depends mainly on the environmental conditions of the new position and so does the survival success of an individual tick.

This concept was also adapted to the ecology research field by August Thienemann ("Wirkungsgesetz der Umweltfaktoren" = effect law of ecological factors) and, relating to the purpose of this thesis, later refined for autecology (= species ecology: study of the interactions of an individual or a single species with the environment) by Tischler (1955): The existence of a species will be determined by those vital factors which are available at least at their minimum (original in German) (Schwerdtfeger 1963).

Relating to Thienemann's law, the plurarity of vital factors influencing a single species can be reduced to just a few "essential factors" (Schwerdtfeger 1963). These factors have been described in tick research before, for the purpose of describing the habitat suitability of *Ixodid* ticks (Wilson 1998): food resources (availability of suitable host for the blood meal) and physical resources (vegetation and associated microclimate). Based on my understanding of ecological effect-relationships and in respect to previous approaches in ecological tick research, I would break the stated physical resources down to the two "essential factors": *temperature* and *humidity* (illustrated as the ticks' "physiological potency" in Figure 2.6 – author`s own graphic and considerations).

The factor *temperature* seems to have a subordinate impact on ticks on the landscape scale, because almost all related studies report decreasing tick abundances with increasing altitude or latitude in general (Materna et al. 2008, Jaenson & Lindgren 2011, Medlock et al. 2013) – which, of course, is a proxy for decreasing mean temperatures. The results of this study portray the same relationship looking at annual averaged values (see Chapter 4.3.1).

Thus, it can be said that *temperature* acts as a limiting factor not only at single study sites but at all areas of low temperatures, high altitudes or northern most expansion.

In addition, the (relative) effect of *temperature* on tick densities is expected to decrease gradually from areas where *temperature* is only just at a minimum available (coldest/highest locations) to areas where the *temperature* is at a tick's physiological maximum. If *temperature* is at optimum, its (relative) effect on tick densities is approaching zero, following considerations of Lundegårdh (1949).

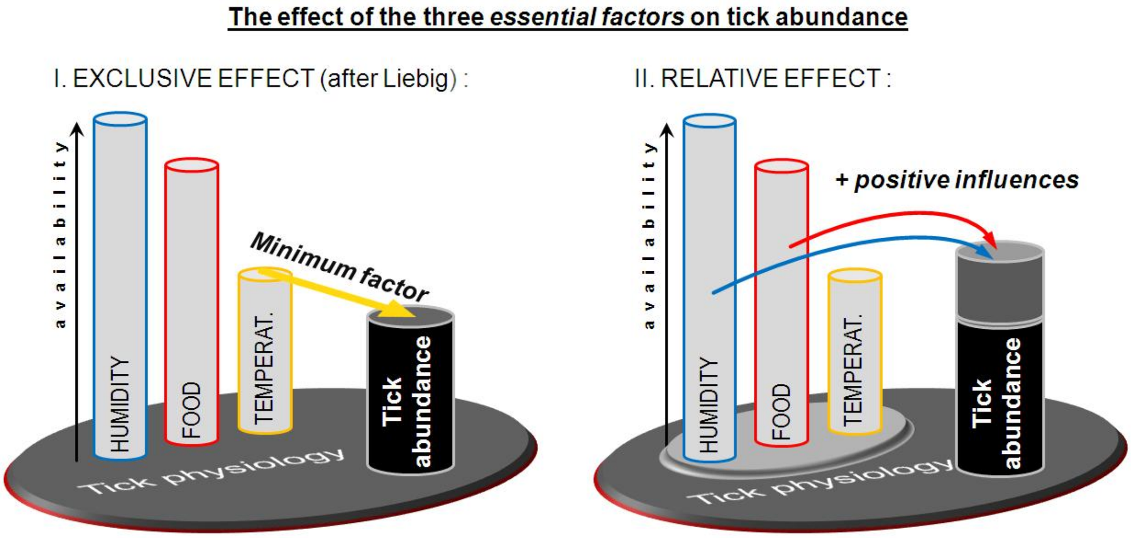


Figure 5.1: Concept of the effect of the three essential minimum factors on tick abundance.

In addition, if the two other "essential factors" affect tick abundances simultaneously, leading to an in- or decrease of the ticks' mortality and thus abundance (in contrast to the limiting definition by Sprengel-Liebig), "relative effect" of *temperature* describes relationships more integrating. Figure 5.1 illustrates the concept. In accordance with the latter, the modeled tick distribution (see Figure 4.10. or Boehnke et al. (2015)) predicts low tick abundances area-wide on the medium mountain ranges *Schwarzwald* and *Schwäbische Alb*, and in colder regions like the *Alpenvorland*. An area-wide impact of other factors can be excluded since the both medium mountain ranges *Schwarzwald* and *Schwäbische Alb* differ strongly in ecological conditions (e.g. precipitation, geology and soils,main forest types.

Therefore, I expect *temperature* to be the only factor which limits tick survival in forest habitats at high altitudes over the entire area on a landscape-scale and on a long-term perspective (but can also act short-term, e.g., by inhibiting questing activity).

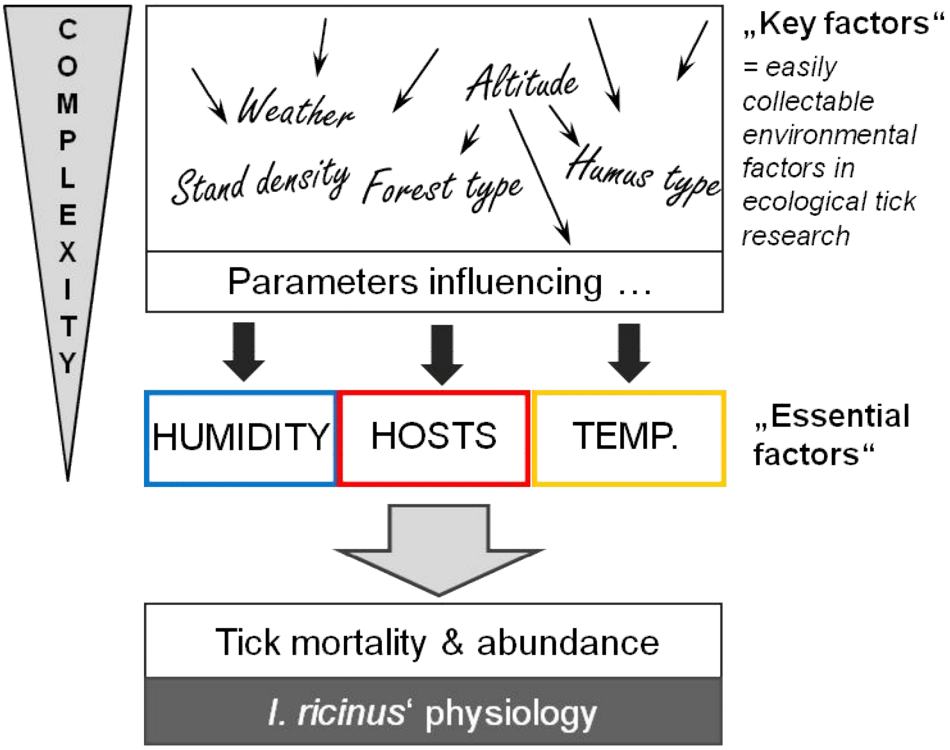


Figure 5.2: The graphic illustrates indirect connections between environmental factors and *I.ricinus* physiology. Tick survival depends mainly on the three "essential factors" *temperature*, *humidity* and *hosts*, so every statistically identified correlation between tick densities and environmental parameters can be linked to one or more of those "essential factors". By identifying the main environmental factors influencing the latter, the tick's habitat suitability should be assessable by a set of basic "key factors".

In contrast, the other two factors *humidity* and *hosts* are expected to act limiting on a smaller scale since conditions change from habitat to habitat. Concluding, even if *hosts* are plentiful, *humidity* factors are suitable and the microhabitat offers adequate shelter – tick abundances will be remarkably smaller in mountainous habitats compared to habitats with the same resources at low altitudes, since *temperature* is near the ticks' physiological minimum and thus has a strong relative effect on the ticks' living processes.

All other factors one records in forest habitats, such as vegetation and soil characteristics, habitat structure etc., influence tick survival rather indirectly by affecting humidity, host and heat availability in the specific habitat (illustrated in Figure 5.2.). I therefore suggest that every statistically significant influence of a superordinate environmental factor on tick densities can be related to one or two of the three "essential factors".

For example, the *humus thickness* or *humus type* is very important for the *humidity* factor (key word: water storage capacity, see Chapter 4.5.3.) and, to a lesser relevant degree, for temperature. To add a little complexity, *frequent rainfall* is expected to equalize differences in the water storage capacity of different humus types (see Chapter 4.5.3.5), so that effects of low water storage capacities (e.g. in mull type humus) should only be noticeable in years with long periods of drought, for instance. In this case, *frequent rainfall* could explain why no effect of the *humus type* on tick densities was observed. Therefore, weather occurrences within the evaluated tick season should be taken into consideration as well.

With this new approach, old and new ecological findings can be interpreted more easily by linking every factor to its main "essential factor(s)". Doing this, we could gather a deeper and more comprehensive understanding of the underlying mechanisms influencing tick abundances from year to year and at different habitats.

5.1.2 SCALES IN ECOLOGY

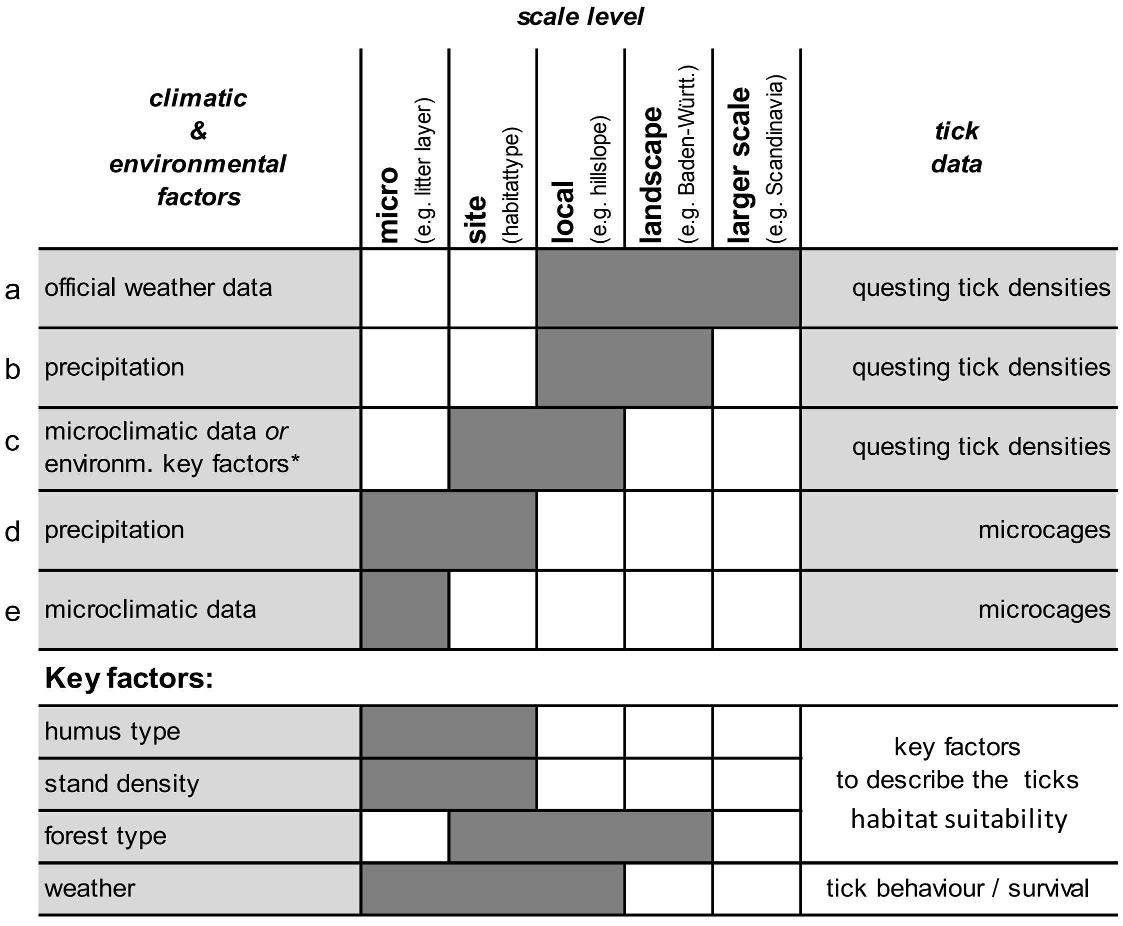
As stated in the previous section, the temperature factor mainly affects tick density distribution in the long-term and area-wide, while the humidity has a more short-term and local effect (e.g., related to the water storage capacity of the humus). Therefore, another concept is needed to put key factors and their underlying processes in an order: the concept of "scales in ecology".

At first, remarks of Simon A.Levin (Levin 1992, page 1944) are used to introduce the problem of scales for ecological tick research:

The problem of scale is “the fundamental conceptual problem in ecology, if not in all of science. Theoretical ecology [..] relates processes that occur on different scales of space, time, and organizational complexity. Understanding patterns” (e.g. the ticks’ density distribution) “in terms of the processes that produce them” (the ticks’ habitat suitability or rather factors influencing the three essential factors *temperature, humidity* and *hosts*) “is the essence of science, and is the key to the development of principles for management. Without an understanding of mechanisms (why, how, when and where ticks are influenced by an environmental factor), “one must evaluate each new stress on each new system de novo, without any scientific basis for extrapolation” (which is, to my opinion, the major problem in ecological tick research); “with such un- derstanding, one has the foundation for understanding and management.”

Many common approaches were used in this thesis to analyse the ecological impact of environmental factors on tick densities and, as a result, many challenges and problems for further interpretations and extrapolations were discovered.
One issue, that was repeatedly raised by the efforts to understand tick density patterns in terms of underlying processes, was: Which data resolutions must be used to interpret the effect of environmental factors on tick densities correctly? On the basis of all findings in this thesis, a set of data combinations was identified that can be attributed to specific research questions. These findings are summarized in Figure 5.3.

In addition, key factors are given. These factors are to be interpreted as “easy to record environmental factors in field research” that can be widely used to describe a ticks’ habitat suitability and to identify their influence on ticks in different situations. It is not intended to be an exhaustive list; in fact, several more uses and research questions could surely be added to each pair of environmental and tick data, and some uses have to be defined more precisely for special usages. However, it is intended to be a intuitive concept helping to improve the conceptual designing of ecological tick research and should be specified in the future.
If we want to examine effects that influence the ticks population abundance (as a rough estimate) or activity in the field, data from tick sampling seem adequate for usage. If the research aims to specify the effect of weather occurrences, characteristics of a ticks microhabitat et cetera on tick survival or mortality, a more specific data sampling approach is needed (e.g. ticks held in microcages with internal sensors measuring microclimatic conditions).



	climatic & environmental factors	***scale level***					***tick data***
		micro (e.g. litter layer)	**site** (habitattype)	**local** (e.g. hillslope)	**landscape** (e.g. Baden-Württ.)	**larger scale** (e.g. Scandinavia)	
a	official weather data			▨	▨	▨	questing tick densities
b	precipitation			▨	▨		questing tick densities
c	microclimatic data *or* environm. key factors*		▨	▨			questing tick densities
d	precipitation	▨	▨				microcages
e	microclimatic data	▨					microcages
	Key factors:						
	humus type	▨	▨				key factors to describe the ticks habitat suitability
	stand density	▨	▨				
	forest type		▨	▨	▨		
	weather	▨	▨	▨			tick behaviour / survival

** e.g. vegetational data and humus type as a proxy for averaged microclimatic conditions*

Figure 5.3: Guideline for the combination of environmental and tick data for ecological tick research at different spatial scales.

The combination of specific climatic and environmental factors with specific tick data are attributed to the following uses:

a → For tick distribution or density modeling (Chapter 4.2) and the forecast of egg-development-dependent distribution boundaries (see Chapter 4.3.3)

b → To evaluate the influence of averaged precipitation conditions on tick densities

c → To estimate the suitability of different forest habitats, the influence of topography on tick densities and behavior etc. (Chapters 4.1, 4.3,. 4.5)

d → To record the influence of precipitation on micro-scaled, short-time humidity conditions ticks are exposed to (Chapter 4.5.3) and their effect on tick mortality

e → To evaluate tick mortality and development as a function of microclimatic average, single weather events etc. (Chapters 4.5.2, 4.5.3)

5.2 SYNTHESIS

Based on the concepts introduced in the last part, findings of this thesis are now combined to a conceptual model for the influence of environmental factors on ticks' living processes and, thus, abundances, on the example of Baden-Württemberg.

The results indicate that the relevance of environmental factors for ticks can change regionally and temporally but, in addition, that there are also some constant pattern, which are mainly driven by the temperature factor. I suggest that there are three zones where the "essential factors" have a different meaning for ticks and, subsequently, also the environmental factors which we record in the field. Figure 5.4 depicts this conceptual model along with relevant factors for each zone.

ZONE A – ZONE OF TEMPORARILY EFFECTIVE FACTORS

Zone A is characterized by a sufficient amount of temperature for the life cycle of *Ixodes ricinus* ticks or, to say it in other words, temperature is near the ticks' physiological optimum. Therefore, *temperature* no longer acts as a long-term limiting factor, its (relative) effect on tick densities approaches zero. Therefore, limiting factors left are *humidity* and *hosts.* The following specifications refer only to the factor *humidity*.

Humidity is expected be a limiting factor that changes locally and annually in its intensity.

On a regional and long-term scale, the humidity in a specific habitat is mainly influenced by the amount of incoming water and the habitat's ability to store this resource: especially average precipitation and the topographic situation. It was, for instance, found that habitats with a high groundwater level such as riparian forests or depressed areas are very suitable for *I. ricinus* ticks due to their constantly available water supply (results in Chapter 4.1.1).

The *humidity* in a tick's sheltering microhabitat, i.e. the humus, was found to be mainly related to two aspects (results in Chapter 4.5.3.). Firstly, the capacity of humus to store water and to withhold emerging water vapor against the dryer atmosphere, which both increase along with the humus thickness/mass. Secondly, the frequency and amount of rain which restores the water supply in the litter layer and soil. The latter is a matter of weather conditions and thus highly variable within years in Baden-Württemberg.

In the following, main aspects for "key factors" are presented.

Suitable humidity conditions in a tick's microhabitat are more likely ..

♣ if the humus is very thick	→ *humus type/thickness*
♣ if the herbal coverage is dense	→ *herbal coverage*
♣ if precipitation is frequent	→ *precipitation*
♣ near water bodies (streams, groundwater)	→ *topography and landscape*

The tick's shelter availability is increased …

♣ if the herb layer densely covers the total area	→ *herbal coverage*
♣ if the humus is thick and covers the total area	→ *humus thickness/coverage*
♣ if they can penetrate the soil body	→ *soil type (grain size)*

Humus is expected to be one of the most important "key factors" to describe a ticks' habitat suitability. However, differences in the humus' storage capacity seem only relevant in times of *rare rainfall* so that effects of the *humus type* on tick mortality and density should only be noticeable in or after long periods of warm and dry weather. In conclusion, the influence of the essential factor *humidity* on tick densities is not permanent but rather changes from year to year due to weather conditions. These conditions are expected to determine whether a key factor (directly influencing humidity) has a major negative (or positive) impact on tick mortality and, subsequently, abundance in a specific season.

A noticeable negative effect of unsuitable humidity conditions on tick densities seems therefore very habitat specific and to occur only occasionally – triggered by meteorological conditions.

ZONE B - TEMPERATURE: A LONG TERM LIMITING FACTOR

Based on all gathered results, I hypothesize that *temperature* acts as the main limiting, "essential factor" at altitudes above 500 m a.s.l. for the example of Baden-Württemberg.

The quantity of heat in a tick's habitat, denoted as temperature within this context, depends mainly on

1) the temperature of the atmospheric air masses (altitude)
2) the amount of directly incoming solar radiation (other key factors)

In general, temperature decreases with height by 0.65 K per 100 m (Mölders & Kramm 2014). The *temperature* near the ground, especially in the litter layer, additionally depends on the amount of directly incoming solar radiation (described in Chapter 2.2.2.). This explains, for example, the differences in egg development at different forest parts depending on stand density and forest type (results in Chapter 4.5.2). This zone is characterized by a total development sum (DdR) at the end of year of about 1.0 at the upper boundary to 2.0 near the lower boundary (results in Chapter 3.3.3).
In the following, main aspects for "key factors" are presented.

The amount of solar radiation warming the ground is reduced …

- ♣ if the forest canopy is compact → *forest stand density*
- ♣ in evergreen coniferous forests → *forest type*
- ♣ near the ground if the humus is very thick → *humus type / thickness*
- ♣ if the mountain slope is exposed to the North → *sun exposure*
- ♣ if the snow cover lasts long in spring → *snow coverage*

Therefore, the mentioned aspects are expected to intensify the limiting power of the *temperature* factor at altitudes above 500 m a.s.l., leading to worse development and activity conditions and, thus, to smaller tick abundances.
These factors can also have a cumulative effect. In a *dense*, *coniferous stand* with a high *humus thickness* the ground warming will be reduced year-long, while in a comparable *deciduous stand* the incoming radiation amount is increased in spring, leading to better conditions for ticks in their season of highest activity. This hypothesis is supported by the results of tick densities found at study sites in Zone B: Coniferous and dense mixed forests stands were in general characterized by low tick densities compared to those found at deciduous forests (Chapter 4.3.1 and 4.5.3).
Concluding, the *temperature* factors seems to have a large-scaled and long-term (over years to decades) influence on tick abundances in areas of high altitude or northern territories, with its most power near the ticks' distribution boundary where *temperature* is only just at a minimum available.

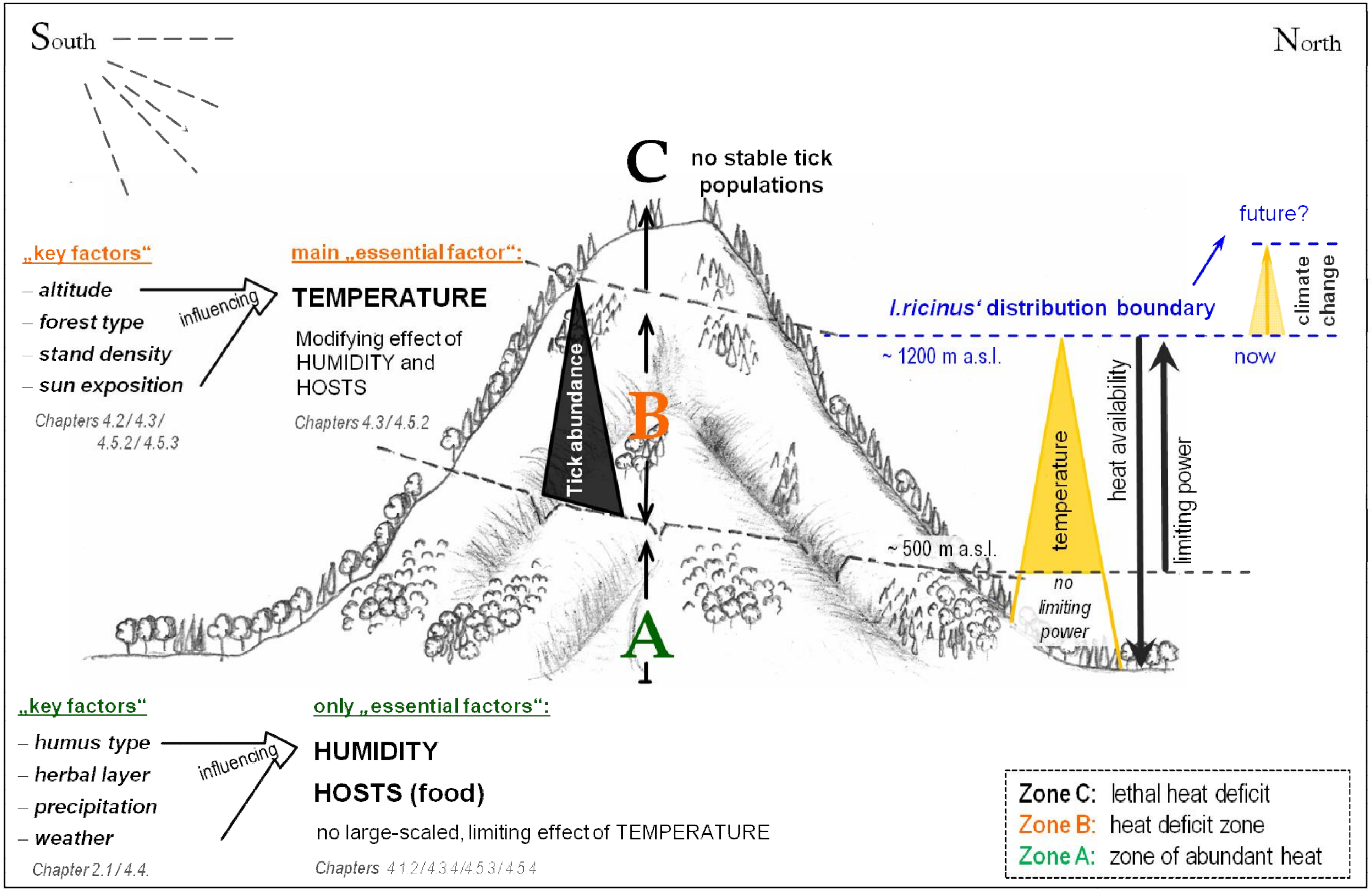


Figure 5.4: Synthesis of the concept of limiting factors and scales in ecology; classifying regions after the impact of environmental factors on *I. ricinus* main physiological needs (expressed as “essential factors”, which are influenced by “key factors” = easily recordable factors to characterize a ticks’ forest habitat).

ZONE C – BEYOND THE TEMPERATURE RELATED TICK DISTRIBUTION BOUNDARY

The highest zone comprises areas above *I.ricinus* distribution boundary. In Baden-Württemberg, only forests at the Feldberg region are currently tick-free and results indicate a distribution boundary of about 1000 to 1200 m a.s.l. with a mean annual temperature of 5 to 6.5°C (results in Chapter 4.1.1). However, a direct transfer of these altitude and averaged temperature values to other regions was found to be limited. In contrast, the use of development rates turned out to be a suitable substitute. An uncompleted egg development (DdR < 1 at the end of year) is suggested to be a good identifier for this zone and can easily be calculated with data from official weather stations (results in Chapter 4.3.3).

Beside this new concept to classify regions by temperature conditions for ticks, this chapter provides another important outcome. Developmental indices calculated on the basis of official weather data could support the selection of study areas in the planning phase of a *Ixodes ricinus* research project with regard to the specific (temperature related) research question.

These findings also provide new information on changes of tick densities along with climate change. Up to now, we know that *I. ricinus* is spreading northwards and to higher altitudes. In addition, Dobson & Randolph (2011) developed a model to examine the influence of environmental factors on tick densities and found that an increase of temperature was especially effective on tick densities in areas where initial climatic conditions are cool or, to give a connection to results from this thesis: in areas were temperature is a limiting factor (= Zone B). That lead to the assumption that in Baden-Württemberg tick densities will mainly increase at altitudes of 500 m a.s.l. and higher in the near future.

6 SUMMARY AND PERSPECTIVES

6.1 SUMMARY

This is the most comprehensive study of microclimate influencing *Ixodes ricinus* abundances in Europe, with respect to the number and ecological variability of study sites continuously sampled over a 2 year period, and also to the continuity and multiplicity of included environmental factors. This is the first time that a set of microclimatic data were recorded on-site and continuously over the entire study period with such a high temporal resolution. In addition, a high variety of analysis approaches was used: partially standard methods in tick research, some methods were tailored from other disciplines and others were devised specifically on the basis of knowledge about the ticks' physiology to suit the purposes. In addition, forests were deconstructed to relevant processes and patterns influencing ticks in their microhabitat.

A total of 25 study sites were examined including 11 deciduous, 8 mixed and 4 coniferous forests, a swamp and a wide clearing, which are distributed over the six largest ecological landscapes and reflect the entire altitude range of Baden-Württemberg. Temperature and relative humidity were measured within the area of ticks' questing activity as well as temperature and humidity conditions in the upper soil and were recorded every 10 minutes. At five so-called Intensive Sites, a plurality of sensors were installed to record the microclimatic variability in forest stands and to compare conditions with the bordering open field. In addition, a multitude of vegetation, humus and soil parameters were recorded particularly with regard to their impact on the microclimate in the ticks' living space. More than 20 vegetation characteristics as well as frequent species were recorded and, afterwards, reduced to a set of eight relevant parameters. Tick data were collected by several project partners using the standard method of monthly flagging from March to October in the period from 2013 to 2015.

The aim of this study was to create a set of useful methods, key factors and concepts which help to identify the suitability of a habitat in its regional setting for *Ixodes ricinus* ticks, on the example of Baden-Württemberg, as an elementary step to identify areas at high risk of a tick bite in the future.

To simplify the high complexity of results and to structure knowledge for further research, I adapted two existing and invented one new concept in the final chapter (conclusive synthesis), with regard to the study aim. Here, only the most important aspects are given for an introduction:

I. Concept of the limiting factor: Based on the Liebig's "Law of the minimum" the variety of environmental factors influencing tick abundances is reduced to three "essential factors": *temperature, humidity* and *hosts.* I hypothesize that the influence of all other statistically determined factors (e.g. forest / humus type) can be directly attributed to their impact on these three *essential factors*, by the knowledge of underlying processes.

II. Concept of scales in ecology: Observed tick pattern are a result of a variety of processes, which operate simultaneously at different spatio-temporally scales. Therefore, to understand the complex interrelationships in the end, we first have to identify the scale on which each process influences ticks. Only by considering these interrelations are we able to improve ecological field research approaches and to get a deeper understanding of tick ecology in the future. As a result, a guideline for the combination of environmental and tick data at different spatial scales is introduced.

III. A conceptual model combines the latter concepts and relevant findings by this thesis. Findings indicate that *temperature* acts limiting on a long-term, area-wide scale near the species distribution boundary, its effect on tick densities gradually decreasing down to warmer areas where the effect of *temperature* approaches zero. Then, tick densities depend mainly on the other two "essential factors" *humidity* and *hosts.*

Chapter 4.1: Spatial and temporal tick density patterns

In the first chapter, the regional context of the study area, tick phenology and density pattern are presented:

(1) The study area of Baden-Württemberg itself is characterized by a high diversity of different landscapes. Each region provides a more or less unique combination of climatic and microclimatic conditions, habitat types, host communities and anthropogenic interventions which all together shape the abundance and activity of the local tick population.

(2) The altitude of the 25 study sites ranged from 111 m to 1288 m a.s.l., the on-site measured mean annual temperature from 4.5 to 11.8°C, the mean annual relative humidity from 81 bis 95% and the mean annual saturation deficit from 0.7 to 2.8 hPa.

(3) There is only a small area left which is above the distribution area of *Ixodes ricinus*, represented by the highest study site *Feldberg*. Results indicate the distribution boundary to be located in between 1000 and 1300 m a.s.l., approximately at about 1200 ma.s.l..

(4) At all other locations, *Ixodes ricinus* ticks were observed. In the years 2013 and 2014, a total of 14564 larvae, 7075 nymphs and 1058 adults were counted. The nymphal densities ranged from 1 to 875 nymphs/year/100 m².

(5) Ticks phenology was varied between years and study sites with a mixture of monomodal, bimodal and indefinite activity pattern, in accordance with other findings from southern Germany. These findings are slightly in contrast to earlier concepts postulating bimodal pattern for central Europe. In fact, a comparison of results from other regions lead to the hypothesis, that activity patterns in central Europe are characterized by a high annual, weather depending variability while tick populations near their distribution boundary show constant activity patterns, presumably evolutionary adaptions to local climatic constraints.

(6) In addition, temperature in spring was very cold in 2013 but very warm in 2014, which lead to an early start and high activity of ticks in spring 2014 compared to spring 2013.

(7) This study showed that tick density not only follows seasonal changes, but can also have a high variability between years. In addition, tick densities were low at study sites of high altitudes and showed a high variability in numbers at study sites at a low or medium altitude level.

(8) The group of study sites with highest nymphal densities, which was determined by hierarchical cluster analysis, contained 85% of the "moist habitats". The results confirm the initial hypothesis that "moist habitats", such as riparian forests or forests located in a depressed area with a high groundwater level, provide very suitable conditions for this tick species. Thus, results confirm common knowledge about the high risk of a tick bite in moist habitats.

(9) However, the tick population at the swamp site was very small indicating that habitats with a very wet ground lead to unsuitable conditions for tick survival or reproduction.

Chapter 4.2: Estimating *Ixodes ricinus* densities on the landscape scale

In the second chapter, a new model approach is presented to estimate the ticks' nymphal densities on a landscape scale, on the example of Baden-Württemberg. The corresponding article already have been published (Boehnke et al. 2015):

(1) Based on a generalized linear model (GLM), the spatial distribution of questing *Ixodes ricinus* nymphal densities was estimated. Results were used to compile high-resolution (grid size: 0.5 km²) maps for the study area Baden-Württemberg. The mapped density of nymphs should be interpreted as the amount of *I. ricinus* nymphs that may be collected by monthly flagging an area of 100 m² in the specific year.

(2) On a landscape scale, official weather data (annual and long-term averages) were the better variable to model tick densities compared to on-site data (annual mean values).

(3) The accuracy of the estimated tick densities was evaluated by leave-one-out cross-validation resulting in root-mean-square errors of 227 nymphs/100 m² (2013) and 104/100 m² (2014).

(4) Low densities of *I. ricinus* are restricted to higher altitudes in the *Schwarzwald* characterised by slightly fragmented coniferous(-rich) forest habitats. Very high densities were estimated for the warmest areas at altitudes below 400 meters. This includes, in particular, the regions along the rivers *Rhine* and *Neckar*, as well as the ambience of *Lake Constance.* Moderate tick densities were estimated for all other hilly countryside with altitudes around 300-800 m a.s.l..

(5) The model gives an overview about the general tick density situation over the study region for a specific year. It is not possible to predict tick densities for the future at present and, of course, tick densities can differ locally from estimated values depending on the specific habitat suitability. However, this is the first map that provides information about the ticks' density for the study region rather than cases of tick borne encephalitis.

Chapter 4.3: Temperature and tick densities

The impact of the temperature factor on tick densities, development and winter mortality:

(1) The temperature has a large-scaled, positive influence on tick densities, leading to decreasing tick numbers with growing altitude. I confirmed these findings using a correlation analysis and linear regression models based on annual data of ticks, temperature and the altitude of study sites.

(2) Tick densities in areas of low altitude showed a high variance in numbers but were constantly low at high altitudes over the years. This finding lead to the hypothesis that temperature has only a strong effect at altitudes of about 500 m a.s.l. and higher. Indeed, the population means of tick densities (all stages) sampled at study sites lower than 500 m a.s.l. differed significantly from those sampled at higher altitudes in both years and, in addition, a correlation between nymphal densities and altitude or mean annual temperatures was found only at study sites of higher altitude according to piecewise linear regression and the additional correlation analysis.

(3) I therefore assume that this threshold describes a break between areas with a strong and subordinate temperature effect on ticks' living processes and thus abundances as a limiting factor and areas where temperature acts not as a limiting factor. This kind of temperature margin was described for the first time within this context.

(4) It was shown that these temperature margins could hamper analysis of further factors influencing tick densities. For example, some studies suggest a generally negative influence of coniferous forests on tick abundances; our results statistically confirm this theory since all coniferous sites are located at high altitudes, however, findings of another

study in Southern Germany prove that coniferous forests are suitable for ticks when they are located at altitudes below 500 m a.s.l..

(5) However, our findings indicate a generally negative influence of the coniferous forest type on tick densities compared to deciduous or mixed stands at high altitudes.

(6) In addition, results indicate that the influence of key factors (e.g. habitat type) on tick populations can be very different in the two zones. This highlights the importance of this temperature margin for further research approaches and leads to the assumption that it is crucial to reconsider the location of the study sites to analyze and interpret results correctly.

(7) There was no evidence that temperature conditions in winter 2013/2014 could have caused the observed reduction in tick abundances all over Baden-Württemberg. But results do actually indicate that the ticks' death due to inoculative freezing is more likely in habitats at higher altitudes and with a low prevention of temperature drops (deciduous forests, little snow cover, thin humus), especially in late winter and early spring.

The newly invented approach using egg development rates to describe the temperature suitability of a habitat lead to four interesting findings:

(8) Egg development could be completed up to 3.3 times at the warmest study site, while it was reduced to 1.4 times at the highest study site with a stable tick population.

(9) The development of ticks is bilaterally restricted at higher altitudes, since the phase of potential development begins later and ends earlier within the activity season.

(10) Study sites at high altitudes showed little differences in the start and end time as well as duration of the temperature-dependent development between years, despite the strong spring temperature differences in 2013 and 2014. In contrast, these weather occurrences remarkably influenced development indices at low to medium altitudes. I therefore hypothesize that the constant conditions lead to stable development and, in conclusion, activity patterns of local tick populations over the years – in areas with a restricted temperature budget (e.g. at high altitudes). This hypothesis is new but can actually help to explain findings retroactively, e.g. from Jouda et al. (2004), who found that the phenology of ticks were stable over the years only at high altitudes.

(11) As expected, the only study site where no ticks were ever found was characterized by an uncompleted egg development (DdR < 1) in both years. Thus, if egg development remains uncompleted within one year, ticks are very likely absent in this area. I therefore hypothesize that this method allows researchers to approximate temperature-dependent distribution boundaries for *Ixodes ricinus* for every northern or mountainous study region in the future and that this approximation by development rates is much more precise compared to the factor altitude or mean annual temperature.

Chapter 4.4: Humidity and tick densities

Analyzing the impact of the humidity factor on tick densities and methodological challenges:

(1) The mean annual relative humidity is relatively high at all study sites, indicating that humidity conditions in Baden-Württemberg's forest habitats are in general suitable for *I. ricinus*, compared to conditions we would expect, e.g., in an Mediterranean region.

(2) In contrast to its physiological meaning, a negative influence of the factor relative humidity (annual mean) on tick densities was calculated ($r = -0.39$). I would explain this by the strong positive influence of the temperature factor ($r = 0.63$), which covers potential causal relationships of ticks with relative humidity and saturation deficit. These results confirm the findings of the GLM approach that on-site measured, microclimatic humidity parameters are not suitable to evaluate the habitats suitability for ticks on a large tempo-spatial scale.

(3) In addition, it was shown that data from official weather stations are not representative for actual moisture conditions ticks are exposed to in a forest habitat (micro- and habitat scale): Scatter plot matrices illustrate well that relative humidity data measured at a nearby official weather station differ strongly from those measured in the forest – this is especially true for the litter and herbal layer in 50 cm height.

(4) Measured values of relative humidity were higher in summer compared to spring in general, reflecting the study regions climate setting with precipitation maxima in summer and winter.

(5) It was observed that, in general, relative humidity increases from outside to inside the forest and from atmosphere to the ground within the forest at all study sites. Conditions in the litter layer showed the strongest deviations from all other heights, staying above the equilibrium humidity of ticks (RH = 85 %) even in spring and summer while, e.g., values of official weather data concentrated below this threshold.

(6) In conclusion, I suggest that humidity-induced, high tick mortality is a matter of temporarily very dry and hot periods or of unsuitable characteristics of the humus type and herbal coverage (following results of the next chapter). It seems, that averaged data can not represent those factors and that several factors related to the moisture availability in the ticks` microhabitat have to be considered simultaneously to estimate its suitability.

Chapter 4.5: Deconstructing forest-microhabitats

Deconstructing forest microhabitats: the effect of vegetation parameters on tick densities and the influence those parameters on temperature and humidity conditions in the ticks' living-space:

(1) Litter depth, litter species and forest type had a significant influence on tick densities in 2013 but not in 2014, indicating that the relevance of those parameters change from

year to year. It was shown how difficult it is to identify environmental key factors based on data of just one or two seasons and how important the knowledge of underlying processes is to interpret results correctly.

Effects of forests stand density:

(2) In 2014, I invented a new approach to test my hypothesis that the factor “forest stand density” has a remarkable influence on temperature-dependent tick development and thus densities at study sites in areas of high altitude (Zone B).

(3) The loggers, located in the litter layer, showed remarkable temperature differences at different forest parts, leading to temporal differences for development completion of about one month.

(4) Results indicate that dense coniferous forest stands reduce the speed of tick development in comparison to lose stands and, in addition, deciduous stands. Since former studies observed a strong reduction of the proportion of hatching eggs with growing altitude, which is a proxy for the available heat amount, I suggest a higher mortality for ticks in heat reduced habitats such as coniferous stands compared to deciduous stand, if located at high altitudes (Zone B).

(5) In addition, these findings confirm the observation that pure coniferous forests have a negative influence on tick densities at high altitudes. The underlying processes can be explained by the effect of the evergreen canopy, which additionally increases the limiting power of temperature for ticks’ temperature-dependent living processes by reducing the ground-near heat amount year-long. In contrast, the forest floor is warmed-up under defoliated deciduous trees in spring, the time of the main tick activity.

(6) I modified some loggers (sensors within with the litter body) and could show a high short-time variance of temperature within the litter body due to radiation induced heating processes. Results indicate that we have to measure directly in a ticks microhabitat (e.g. inside the microcages, when testing for ticks’ mortality) to record actual conditions ticks are exposed to.

Litter layer and humus type:

(7) The litter layer is one of the most important and, at the same time, unstudied microhabitats of *I. ricinus*. Each litter (and its resulting humus type) is mainly a product of the vegetation mixture and its decomposition and therefore highly variable space (and time), which could have been a major obstacle for an integration in former tick research.

(8) Aware of this problem, I reduced complexity to the main issue: the humus’ suitability for tick’s living processes, expressed as its influence on the ticks’ *essential factors* temperature and humidity. Consequently, two main humus types are differentiated: constantly and thick humus (moder / raw humus) and, in contrast, the mull humus which is

characterized by a shrinking humus mass in the warm seasons, leading to reduced shelter for ticks.

(9) In addition, there are two different situations related to the "amount of solar radiation reaching the litter layer" which have to be separated. First, a highly reduced amount attributed to a dense, foliated forest canopy and, second, a rarely reduced amount attributed to a defoliated or very fragmentary canopy. The latter directly leads to humus warming (absorption) and occurs mainly in deciduous forests in spring and in loose stands.

(10)In general, results confirmed former findings from quasi-natural approaches that the humus' thickness has a remarkable influence on its internal humidity (Park et al. 1998).

(11)Under thick humus, humidity stayed at higher levels and temperature more constantly compared to thin humus – especially in summer when precipitation was frequent. Additionally, it was found that thick humus can maintain high humidity levels longer than thin humus, related to their water storage capacity. I therefore conclude that especially in warm and dry periods the humus type is crucial for tick survival.

(12)I therefore suggest that the key characteristics of a humus, related to its ability to maintain suitable humidity conditions for ticks, is its amount of actual stored water and its ability to withhold the emerging water vapor against the dryer atmosphere.

(13)Results indicate that the humus type can have a local influence on ticks activity start: thick humus masses reduce the heating effect of solar radiation near the forest floor (especially in spring when the radiation energy is low) and could therefore delay the temperature-dependent awakening of overwintering ticks. In addition, thick humus masses can store a high amount of liquid water which additionally reduces the radiation-induced heating of the humus via evaporative cooling.

(14) Results of this study indicate a strong negative effect of clay-rich soils combined with a sparse litter and herbal layer on the moisture availability for ticks at periods of long drought (Kirchheim Teck, summer 2013) and thus on tick abundances, respectively. Here, the moisture factor became limiting due to weather occurrences, which were not buffered by, e.g., a thick litter layer with a higher moisture retention capacity.

(15)A remarkable negative effect of unsuitable humidity conditions on tick densities seems therefore very habitat specific and to occur only occasional – triggered by meteorological conditions.

Chapter 5: Conclusive synthesis

Conclusive synthesis and introduction of useful ecological concepts for tick research:

(1) The "Minimum law" of Sprengel-Liebig was used to reduce the variety of environmental factors that influence ticks to the "essential factors": *temperature, humidity* and *hosts*.

(2) I hypothesize that all other statistically determined factors affecting tick abundances (e.g. forest/humus type) can be attributed to their impact on these three *essential factors*. With this new approach, old and new ecological findings can be interpreted more easily by linking every factor to its main *essential factor*(s).

(3) “Key factors” have a strong and direct influence on a “essential factor”, e.g., the humus type and precipitation on *humidity*. These “key factors” can easily be applied to identify a habitats suitability for ticks (in general or related to weather events). Some “key factors” are presented, their assignability to other regions has to be verified.

(4) The concept of “essential and key factors” is embedded in a greater context with the concept of “scales in ecology”, which attributes to the different scales of space, time and complexity specific processes occur in nature.

(5) Thus the question was raised: “Which data resolutions must be used to interpret the effect of environmental factors on tick densities correctly?” As a solution, a guideline for ecological tick research is worked out. This list of suitable data combinations is based on findings in this thesis. It is not meant to be complete, but should give a first starting point to standardize ecological tick research approaches in the future.

(6) Furthermore a conceptual model on the influence of environmental factors on tick abundances was developed. It combines the concepts of “essential and key factors” and “scales in ecology” and integrates related findings of this thesis for the example of Baden-Württemberg. I suggest that this concept, if frequently used, leads to a deeper and more comprehensive understanding of the underlying mechanisms influencing tick abundances from year to year and in different habitats in the future.

(7) Findings indicate that the factor *temperature* acts limiting on a long-term, area-wide scale near the species boundary of about 1200 m a.s.l. at present, its effect on tick densities gradually decreasing down to about 500 m a s. l.. At warmer areas, the effect of *temperature* approaches zero and tick densities depend mainly on the other two “essential factors”. However, even if *temperature* acts as the main limiting factor, *humidity* and *hosts* can affect tick densities simultaneously (→ relative effect of *temperature*).

(8) In contrast, results indicate that the factor *humidity* (“moist habitats” excluded) mainly has a short-term, weather-driven effect on tick abundances. Its effect changes from year to year and habitat to habitat –depending on key factor characteristics (e.g. humus type).

Concluding, results and considerations demonstrate well the complexity of the matter and how difficult it is to identify responsible factors affecting tick densities. A plurality of single findings was generated in the past, and some more are added by this thesis, which represent pieces of the large puzzle of environmental factors affecting *I. ricinus* populations. However, one of the main issue in tick ecological research is the poor spatial and temporal transferability of these findings.

My study showed that the application of suitable concepts for field study design and a more holistic integration of resulting findings is essential to create a scientific basis for extrapolation, deeper understanding and management. Therefore it seems essential to develop specific standards for ticks' field research in Europe and the subsequent data analysis in the future. Close attention should be paid on the detailed examination of the ticks' microhabitat and its influence on the ticks' physiologically-essential factors in respect to the spatial embedding of the habitat.

6.2 FUTURE WORK

Results of this study indicate that the coniferous forest type has a remarkable negative influence on tick densities at high altitudes and in areas where temperature acts as a long-term limiting factor, respectively. In Baden-Württemberg, the medium mountain range *Schwarzwald* is currently dominated by pure coniferous forests. However, changing forest practices aim to a higher proportion of deciduous trees and more ecologically valuable stands, which could promote higher tick densities in the future. Further, climate change is expected to reduce the limiting effect of temperature in this area additionally, which would increase tick densities even more. Thus, further long-term studies on the impact of temperature-related factors on tick abundances are highly recommended in the *Schwarzwald* using my results as a reference. Additionally, public authorities should prepare for those changes and increase efforts in prevention measures, for example, by raising public awareness.

It is well known, that weather conditions can have a remarkable influence on the ticks' mortality, abundance and activity (Sixl & Nosek 1971, Randolph & Storey 1999, Dautel 2010). In this study, the intensity of the influence of specific, habitat ecology related factors on tick densities was found to vary between years and locations. It is expected, that mainly weather conditions are responsible for this changes: Humus characteristics control the ticks' humidity supply but are in turn strongly affected by the frequency of rainfall, for instance. The integration of weather pattern and their impact on tick mortality was beyond the scope of this thesis, but could provide a more detailed and complete view of the influence of single key factors on ticks within this or, of course, other study areas. This knowledge is the basis for a more precise prediction of tick abundances in a following year or even tick activity pattern in the future. Thus, a study that examines the impact of weather pattern on ticks is recommended.

The effect of snow cover on tick mortality is one part of tick research that has not gained much attention yet. Until now mainly positive aspects, like the sheltering effect that preserve ticks from freezing temperatures, have been discussed (Dautel 2010). In the opinion of the author, long lasting snow coverage likely intensifies temperature related problems at higher altitudes, since it delays the start of tick development and activity in spring. Beyond that, melting snow could cause a more highly death probability due to the drowning of ticks. Further examinations on the ticks' winter mortality should include these new aspects.

The aim of the study was to provide a set of useful methods, key factors and concepts for further ecological tick research, especially for a better estimation of areas or habitats suitability for ticks in the field. It would be most helpful to use and verify the newly invented method based on development rates to predict *I. ricinus* temperature-related distribution boundaries (see Chapter 5.2) in other study regions of high altitude or far north. In addition, more long-term studies on the effect of introduced key factors are needed to specify the latter and identify exceptions for this and other study regions. Thus, the use and successive improvement of the presented concepts and the guideline for tick ecology research is highly recommended.

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LIST OF ABBREVIATIONS

a.s.l.	Above sea level
BW	Baden-Württemberg
DWD	German Weather Service (Deutscher Wetterdienst)
DdR	Daily development rates
GLM	Generalized linear model
K	Kelvin
KIT	Karlsruhe Institute of Technology
LGA	BW State Health Office (
max	Maximum
min	Minimum
mT_A	Mean annual air temperature
n	Number of samples
ND	Nymphal density
NDVI	Normalized Difference Vegetation Index
RH	Relative humidity
SD	Saturation deficit
SM	Soil moisture
TBE	Tick-borne encephalitis
T_A/Ta	Air temperature
T_L/TL	Litter temperature
T_s/Ts	Soil temperature

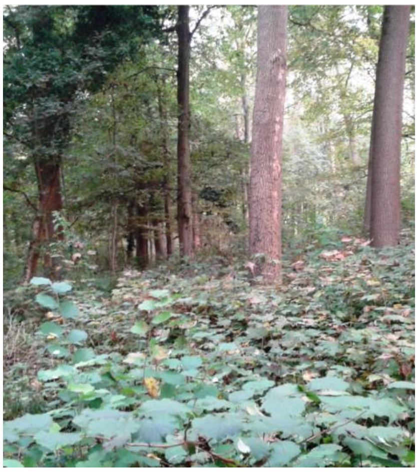
APPENDIX A

STUDY SITES

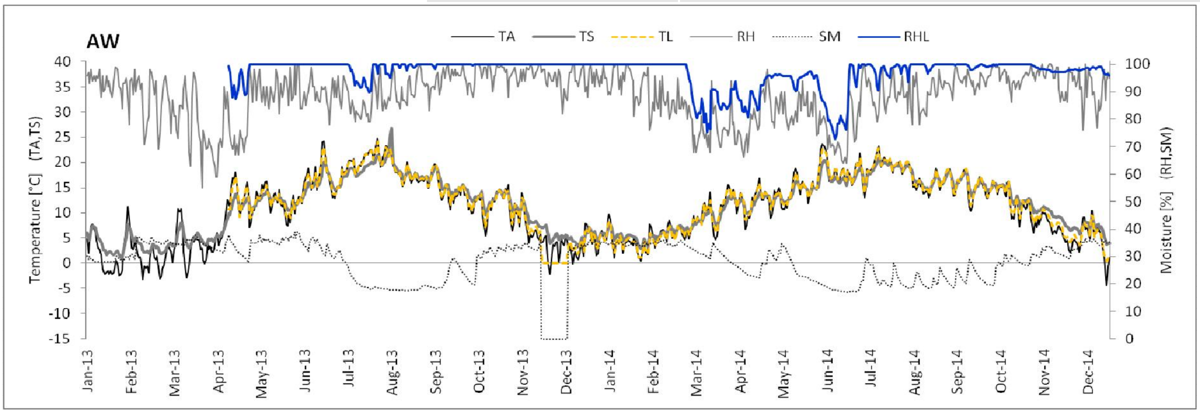
INTENSIVE SITES

Auwald (AW)

Oberrheintiefland 111 m a.s.l.



Lon / Lat	8,3765 / 49,1338
Forest type	Deciduous forest (riparian forest)
Crown density	medium
Herbage incidence	medium
Herbage coverage	fragmentary
Litter depth	low
Litter coverage	fragmentary
Soil / humus type	Vega, mull
Comments	A seldom flooded riparian forest, located in the broader vicinity of the river *Rhine*. Species-rich tree composition of different ages, old oaks and groups of young maple in places. Little leaf litter remained at the end of summer. Sings for high bioturbation (no humic A-horizon).



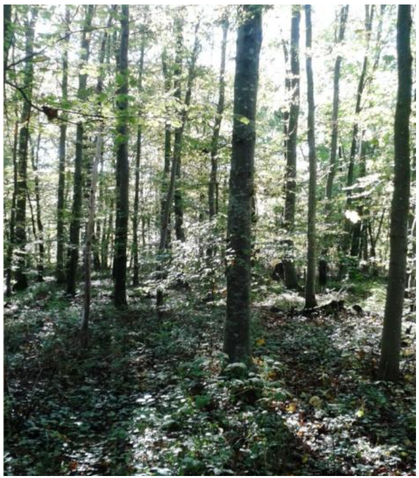
TA = air temperature, TS = soil temperature, TL = litter temperature

RH = relative humidity (air), SM = soil moisture, RHL = relative humidity (litter)

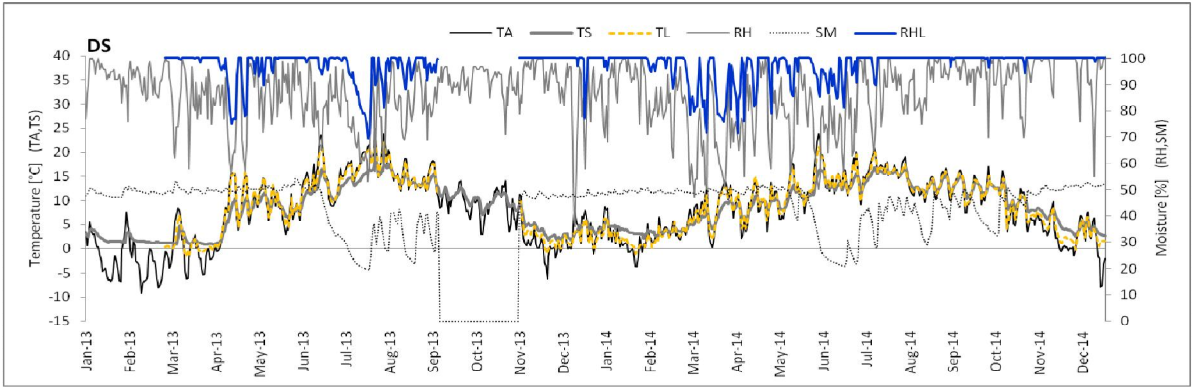
Drackenstein (DS)

Schwäbische Alb

755 m a.s.l.



Lon / Lat	9,6562 / 48,5566
Forest type	Deciduous forest
Crown density	medium to high
Herbage incidence	medium to high
Herbage coverage	continuous
Litter depth	medium to small
Litter coverage	fragmentary
Soil / humus type	rendzic leptosols (WRB), mull
Comments	Medium dense forest stand with high deciduous tree species diversity on a thin, stony layer of soil. Medium to small shrub cover. Located at the western border of a partly fragmented forest area, bordering to agricultural land.

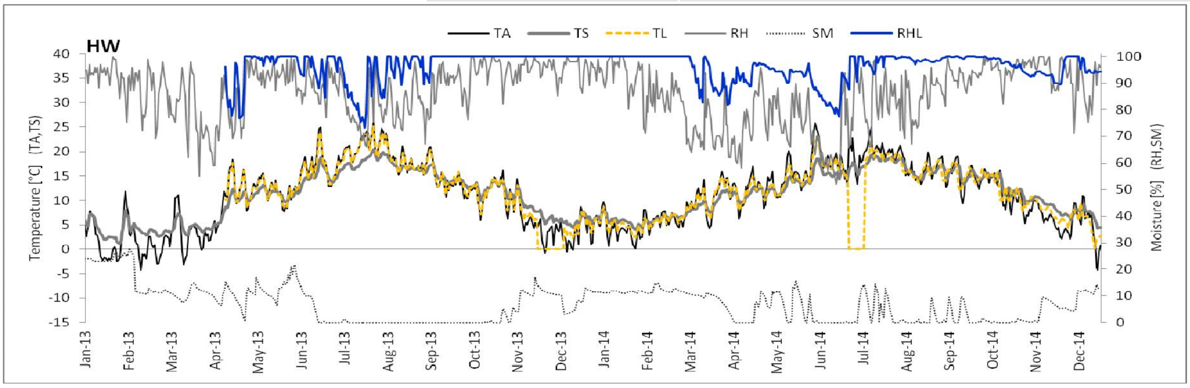


Hardtwald (HW)

Oberrheintiefland

117 m a.s.l.

Lon / Lat	8,4798 / 49,1338
Forest type	Deciduous forest / Coniferous forest
Crown density	medium to high
Herbage incidence	medium to small
Herbage coverage	fragmentary
Litter depth	medium
Litter coverage	continuous
Soil / humus type	cambisol (WRB), moder
Comments	Timberland, a part beech and a part pine dominated, located on nutrient-poor, water permeable sandy soils. Ticks were sampled only in the beech stand, with a poor shrub and herbage cover but a dense humus layer. Located at the western border of a continuous forest area, surrounded by agricultural land.

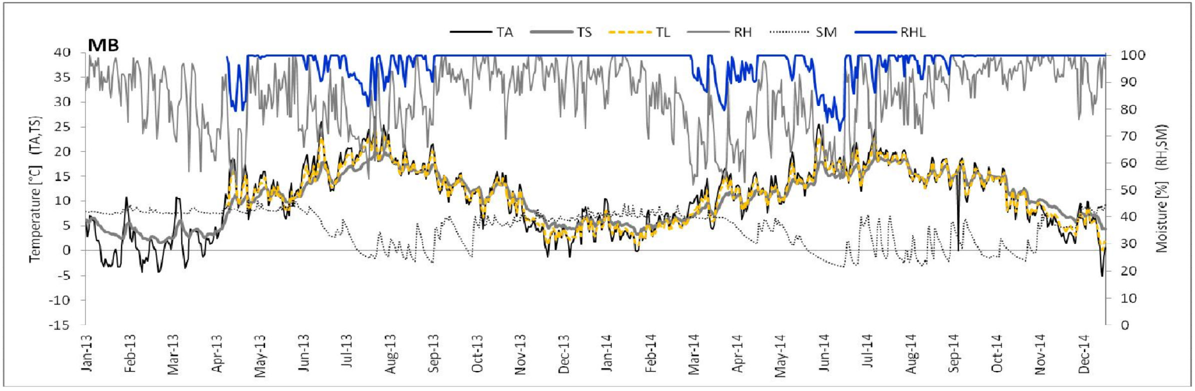


Michaelsberg (MB)

Oberrheintiefland / N.-T.-Gäuplatten

253 m a.s.l.

Lon / Lat	8,5726 / 49,0882
Forest type	Deciduous forest
Crown density	medium to high
Herbage incidence	medium
Herbage coverage	fragmentary
Litter depth	medium to high
Litter coverage	continuous
Soil / humus type	Pararendzina, moder to raw humus
Comments	Beech dominated, dense forest stand with some old and large trees towering above the upcoming younger trees of > 3m height. A mosaic of low to high shrub and herbage cover, medium incidence of lying deadwood and plenty earth worm feces on the thick soil and humus layer. Intensive Station is located on a wooded, formerly farmed terrace.

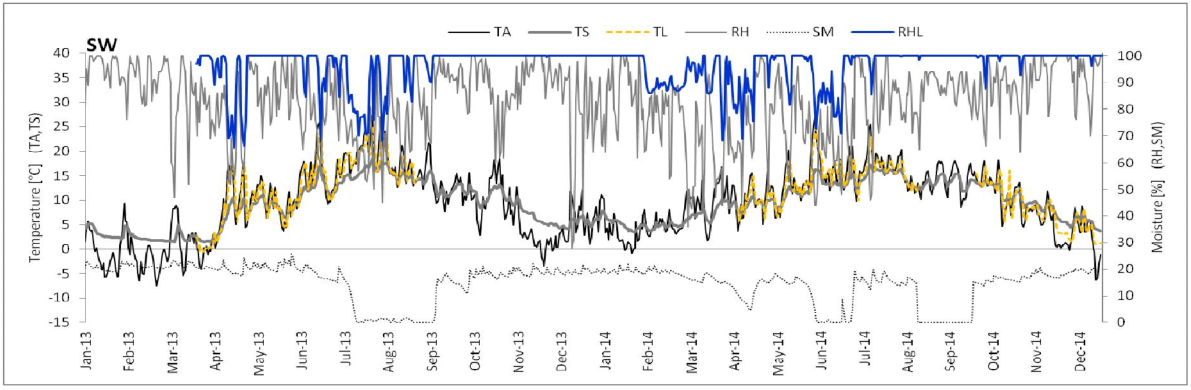


Schwarzwald (SW)

Schwarzwald

610 m a.s.l.

Lon / Lat	8,4425 / 48,7740
Forest type	Mixed forest
Crown density	medium to high
Herbage incidence	small
Herbage coverage	continuous
Litter depth	medium
Litter coverage	continuous
Soil / humus type	Regosol (WRB), raw humus
Comments	Forest mainly made up by beech, spruce and fir trees located within the large, continuous forest areas of the northern Schwarzwald. A pasture borders to the south,



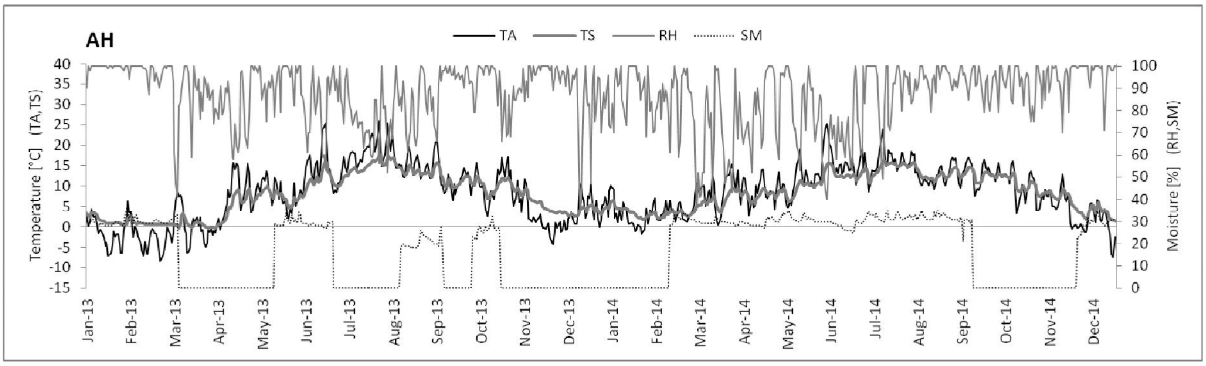
BASIC SITES

Allerheiligen (AH)

Schwarzwald

773 m a.s.l.

Lon / Lat	8,1814 / 47,8623
Forest type	coniferous forest
Canopy density	medium to high
Herb layer density	low to medium
Herb layer coverage	fragmentary
Litter depth	medium
Litter coverage	continuous (needle), patchy (beech)
Soil / humus type	podzol (WRB), raw humus
Comments	Coniferous forest with single beech trees. Little to medium shrub cover. Distinct signs for rodents (plenty of tunnels, bite marks at equipment), but not for deer (young white firs show no bite damages). Acidification indicators (blueberry). Surrounded by large continuous coniferous and mixed forest areas.



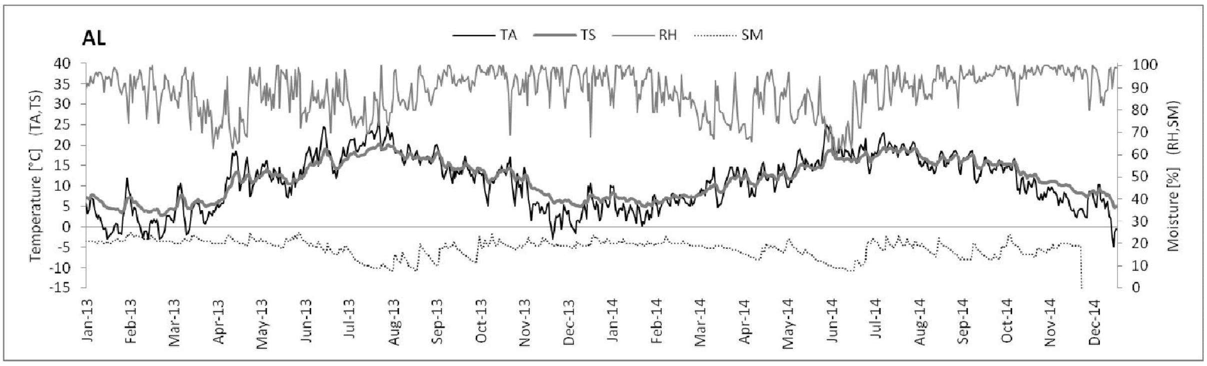
TA = air temperature, TS = soil temperature,

RH = relative humidity (air), SM = soil moisture

Altenheim (AL)

Oberrheintiefland 147 m a.s.l.

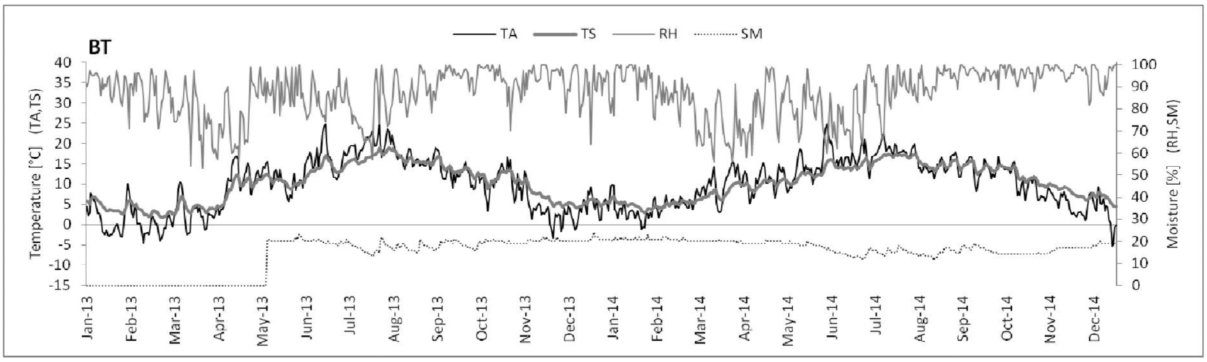
Lon / Lat	7,7796 / 48,4693
Forest type	deciduous forest (riparian forest)
Canopy density	medium
Herb layer density	medium
Herb layer coverage	fragmentary
Litter depth	low to medium
Litter coverage	fragmentary
Soil / humus type	Kalkpaternia, mull
Comments	A frequently flooded riparian forest located at an old sidearm of the river *Rhine*. High shrub cover. Little leaf litter remained at the end of summer, Distinct sings for high bioturbation (plenty of earth worm feces, no humic A-horizon). Mounds of burrowing animals are common.



Botnang (BT)

Schwäbisches Keuper-Lias-Land 349 m a.s.l.

Lon / Lat	8,1814 / 47,8623
Forest type	mixed forest
Canopy density	medium to high
Herb layer density	medium
Herb layer coverage	fragmentary
Litter depth	medium
Litter coverage	continuous, single patches of rarely litter
Soil / humus type	vertisol cambisol (WRB), mull-like moder
Comments	Timberland characterized by areas of different tree composition. Dominant is beech or common spruce, other species grows loose in between. Distinct sings for high roe deer (young spruces show heavy bite damages) and for small wild boar abundance (despite lots of acorns no signs of rooting). The forest is surrounded by settlements and infrastructure of the capital city Stuttgart.

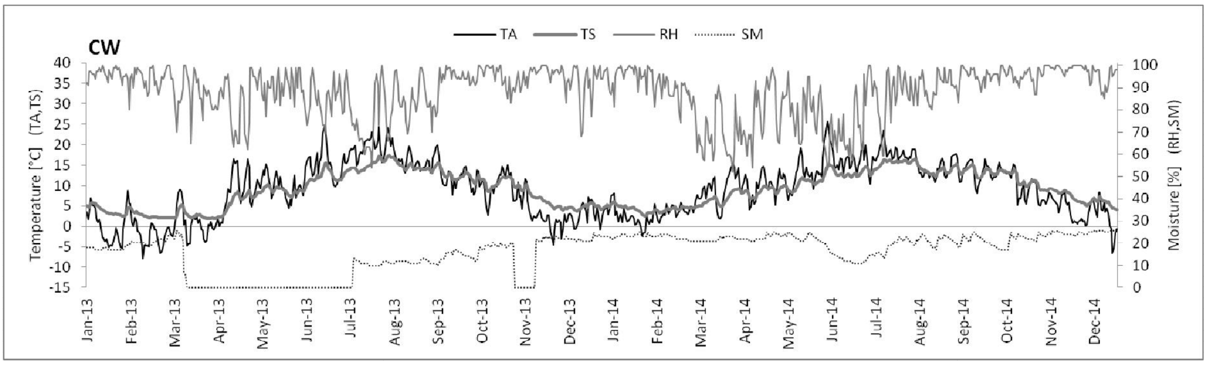


Calw (CW)

Neckar-Tauber-Gäuplatten

532 m a.s.l.

Lon / Lat	8,8278 / 48,7419
Forest type	mixed forest
Canopy density	high
Herb layer density	low
Herb layer coverage	continuous
Litter depth	high
Litter coverage	continuous
Soil / humus type	rendzic leptosols (WRB), raw humus-like moder
Comments	Beech dominated timberland with pine and common spruce. Little shrub cover. North-facing slope. Located at the northern border of a large continuous forest area, surrounded by agricultural land.

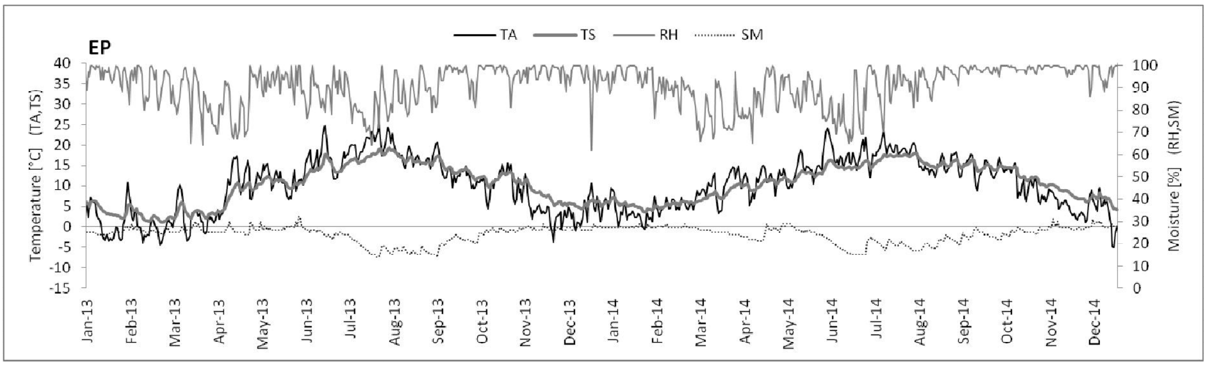


Eppingen (EP)

Neckar-Tauber-Gäuplatten

268 m a.s.l.

Lon / Lat	8,9260 / 49,1107
Forest type	deciduous forest
Canopy density	medium
Herb layer density	medium
Herb layer coverage	continuous
Litter depth	medium
Litter coverage	continuous
Soil / humus type	vertisol cambisol (WRB), moder
Comments	Forest stand with high deciduous tree species diversity and single coniferous trees. Medium shrub cover. Located near the western border of a large continuous forest area, surrounded by agricultural land.

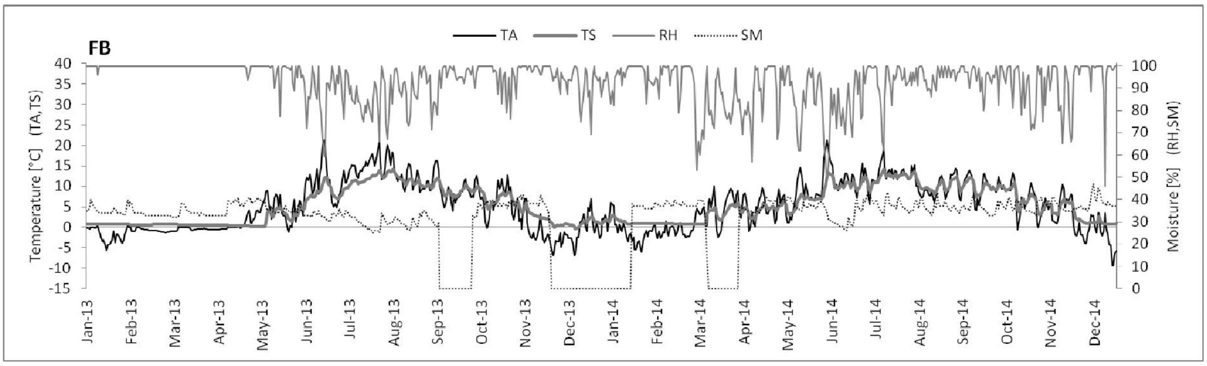


Feldberg (FB)

Schwarzwald

1288 m a.s.l.

Lon / Lat	8,0382 / 47,8623
Forest type	coniferous forest
Canopy density	medium to small
Herb layer density	medium
Herb layer coverage	continuous
Litter depth	medium
Litter coverage	continuous
Soil / humus type	NA, raw humus
Comments	Spruce and fir dominated forest, with single beech trees (dwarfish growth). Long periods of snow cover in winter are typical. Litter is made up by needles, beech (fragmented) and Herb layer remains. Acidification indicators (blueberry). Surrounded by large continuous coniferous and mixed forest areas.

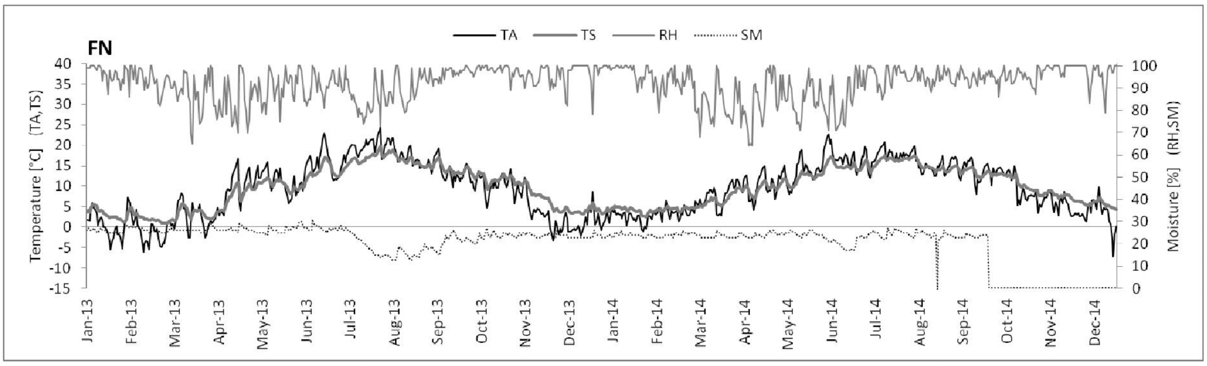


Friedrichshafen (FN)

Alpenvorland

425 m a.s.l.

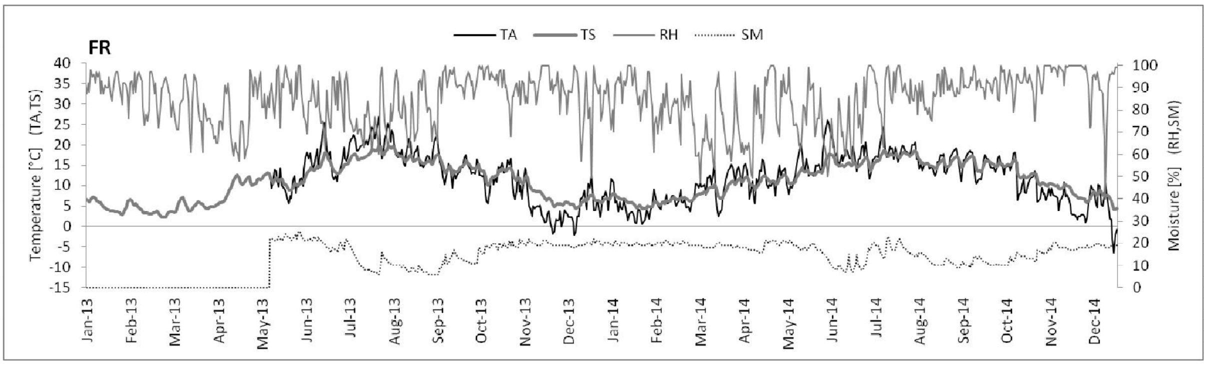
Lon / Lat	9,5121 / 47,6515
Forest type	mixed forest (moist forest)
Canopy density	medium to high
Herb layer density	medium to small
Herb layer coverage	fragmentary
Litter depth	high
Litter coverage	continuous, single patches of rarely litter
Soil / humus type	cambisol (WRB), mull-like moder
Comments	Moist forest with high tree species diversity near *Lake Constance*. Litter is made up by beech, oak and other leave species. Sinks with moisture indicators (*Juncus spp*, black alder), a little stream flows near the ground surface. Near the western border of a wide forest area. Settlements border to the west.



Freiburg (FR)

Oberrheintiefland / Schwarzwald 382 m a.s.l.

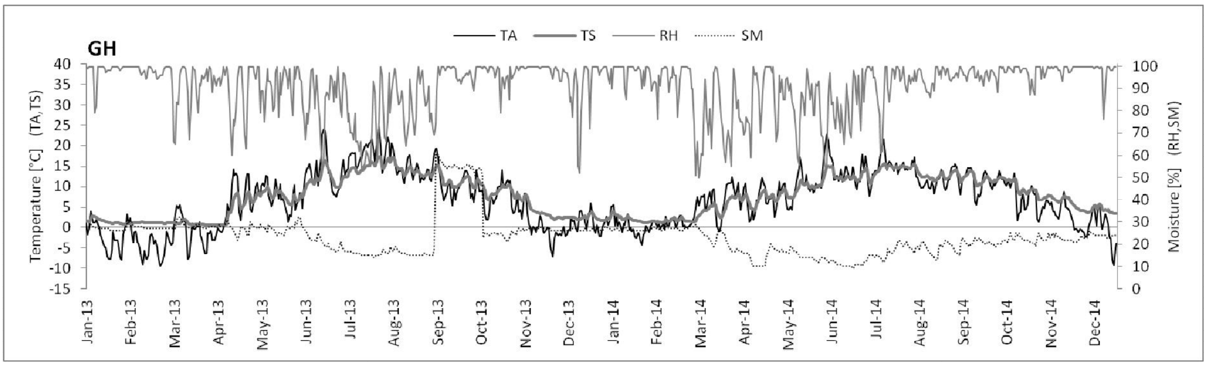
Lon / Lat	7,8816 / 47,9962
Forest type	deciduous forest
Canopy density	medium to small
Herb layer density	medium to small
Herb layer coverage	fragmentary
Litter depth	medium
Litter coverage	continuous, single patches of rarely litter
Soil / humus type	NA, moder
Comments	Deciduous forest with southern sun exposition, single coniferous trees and a high incidence of lying deadwood. Located near the southern border of the large continuous forest areas of the *Schwarzwald* region.



Gosheim (GH)

Schwäbische Alb 992 m a.s.l.

Lon / Lat	8,7600 / 48,0999
Forest type	mixed forest
Canopy density	high to small
Herb layer density	high to small
Herb layer coverage	fragmentary
Litter depth	medium to small
Litter coverage	fragmentary; substitute: moss
Soil / humus type	regosol (WRB), moder
Comments	The second highest study site is characterized by a medium-dense, coniferous dominated mixed forest stand containing several clearance patches. The forest floor is mainly covered by moss, patches of dense grass cover occur as well.

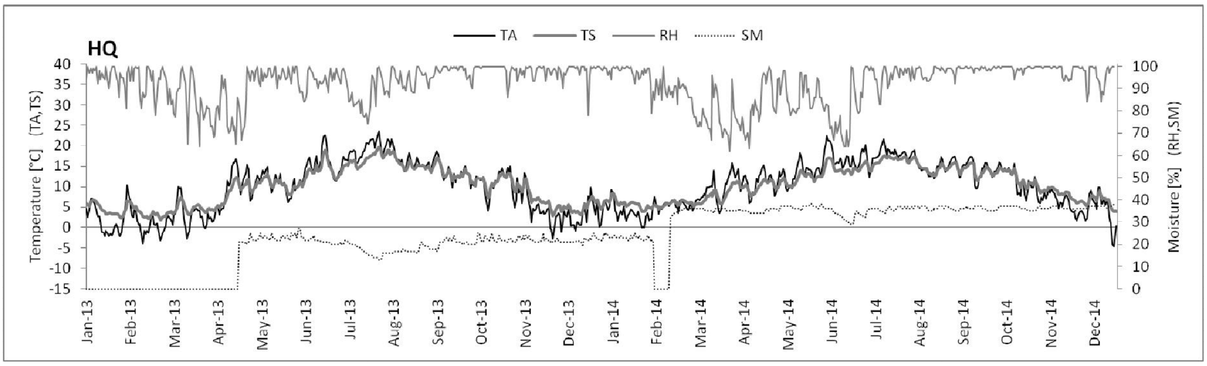


Hedwigsquelle (HQ)

Oberrheintiefland / Schwarzwald

215 m a.s.l.

Lon / Lat	8,4356 / 48,9545
Forest type	deciduous forest
Canopy density	high
Herb layer density	low
Herb layer coverage	fragmentary
Litter depth	high
Litter coverage	continuous
Soil / humus type	NA, moder to raw humus
Comments	Forest mainly made up by young beech trees, growing atypically at the slopes of a ravine in the western extensions of the *Schwarzwald*. A little stream flows at the ravine bottom most of the year. It is characterized by conditions which are cooler and more damp compared to its surroundings.

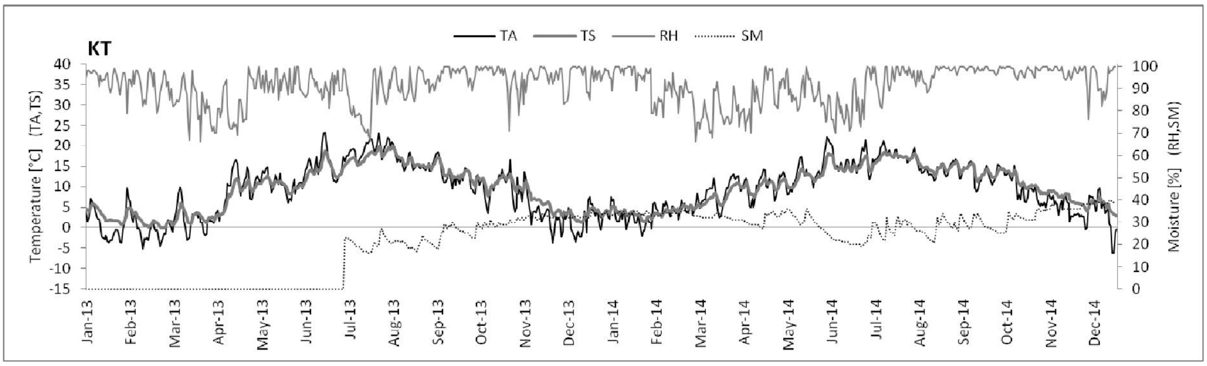


Kirchheim unter Teck (KT)

Schwäbisches Keuper-Lias-Land

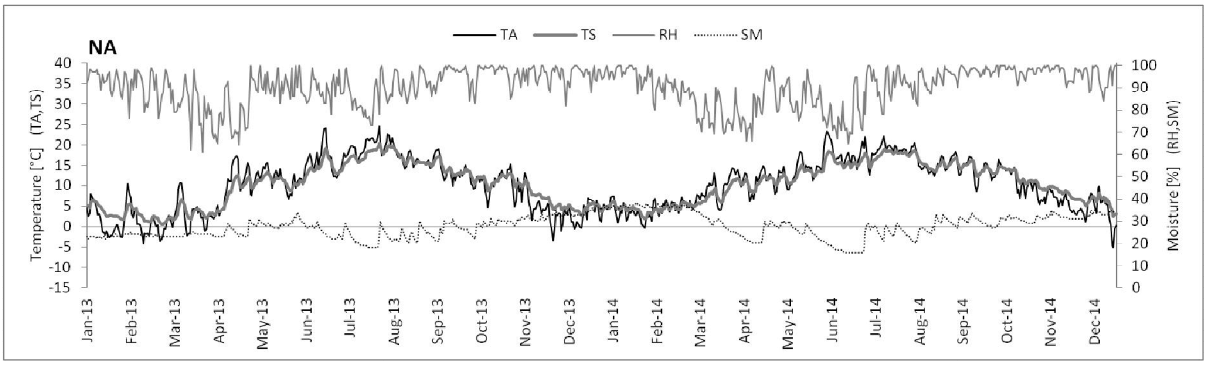
337 m a.s.l.

Lon / Lat	9,4284 / 48,6286
Forest type	deciduous forest
Canopy density	medium to high
Herb layer density	low
Herb layer coverage	fragmentary
Litter depth	low
Litter coverage	continuous
Soil / humus type	Pseudogley-Pelosol, mull
Comments	No dominating tree species. Litter was nearly exhausted at the end of summer 2014 and 2015. The clay-rich soil showed deep cracks after longer periods of drought. Distinct sings for high bioturbation (plenty of earth worm feces, small humic A-horizon). Near the northern border of a wide forest area, surrounded by agricultural land. Intensely used for recreational activities.



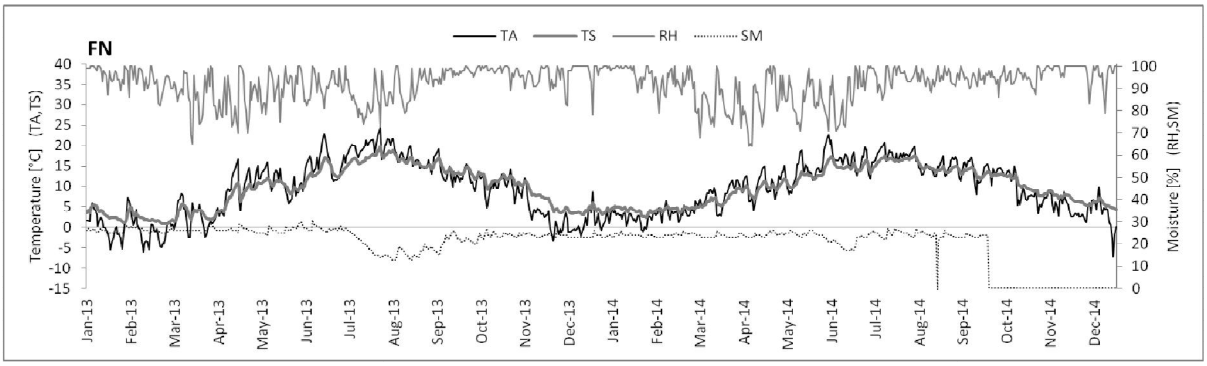
Neckaraue (NA) *Neckar-Tauber-Gäuplatten* 204 m a.s.l.

Lon / Lat	9,2060 / 48,9862
Forest type	deciduous forest
Canopy density	medium
Herb layer density	medium to small
Herb layer coverage	continuous
Litter depth	medium to small
Litter coverage	continuous
Soil / humus type	Gley-Kolluvisol, mull
Comments	The forest is part of the nature protection area “Unteres Tal/Haldenrain”, located along the banks of the river Neckar. A small foodpath leads in this shrub rich riparian forest. Distinct sings for high bioturbation (plenty of earth worm feces, small humic A-horizon). Mounds of burrowing animals and edible snails are common.



Parzelle Hohenheim (PH) *Schwäbisches Keuper-Lias-Land* 472 m a.s.l.

Lon / Lat	9,0898 / 48,6801
Forest type	mixed forest
Canopy density	medium to high
Herb layer density	low
Herb layer coverage	fragmentary
Litter depth	medium
Litter coverage	continuous to fragmentary
Soil / humus type	mull-type moder
Comments	Site with tick activity parcels. The study area is located in the middle of a large forest characterized by patches of dense spruce timberland as well as mixed stands with different species compositions, mainly beech, hornbeam and pine. Distinct sings for high bioturbation (plenty of earth worm feces, small humic A-horizon) and rooting wild boar under mixed stands.

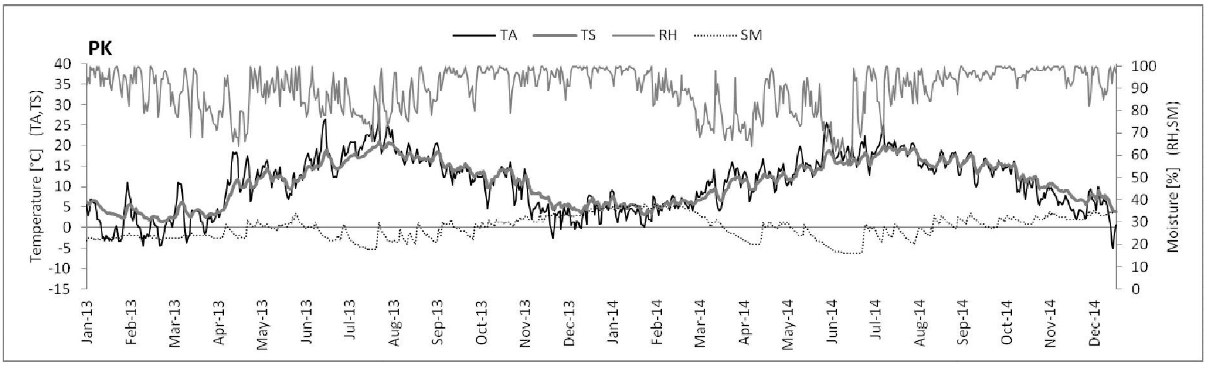


Parzelle Karlsruhe (PK)

Oberrheintiefland

126 m a.s.l.

Lon / Lat	8,4257 / 49,0280
Forest type	mixed forest
Canopy density	medium to high
Herb layer density	small
Herb layer coverage	fragmentary
Litter depth	medium
Litter coverage	continuous, single patches of rarely litter
Soil / humus type	moder
Comments	Site with tick activity parcels. Beech and pine as main tree species. High incidence of lying dead-wood and distinct signs for wild boar (large areas of rummaged ground). Located at the eastern border of the urban forest of Karlsruhe, frequently used for recreational activities.

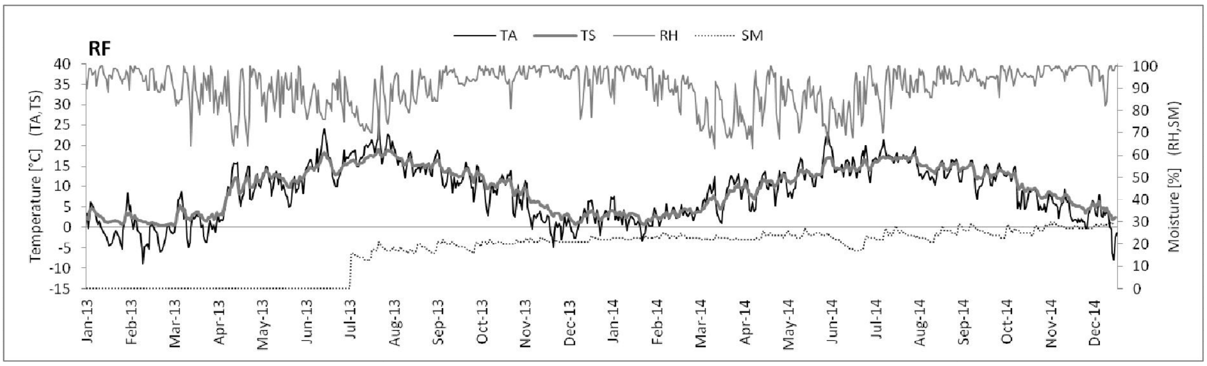


Rosenfeld (RF)

Schwäbisches Keuper-Lias-Land

512 m a.s.l.

Lon / Lat	8,7241 / 48,3219
Forest type	clearance
Canopy density	none
Herb layer density	high
Herb layer coverage	continuous
Litter depth	NA
Litter coverage	NA; remnants of grass vegetation
Soil / humus type	NA
Comments	Wide clearance, at the eastern border of a large forested area. The ground is covered by continuous, non-cutted grass vegetation. The soil is of a strong red, clay-rich and has no humic A-horizon. Single, young pine trees are near the logger.

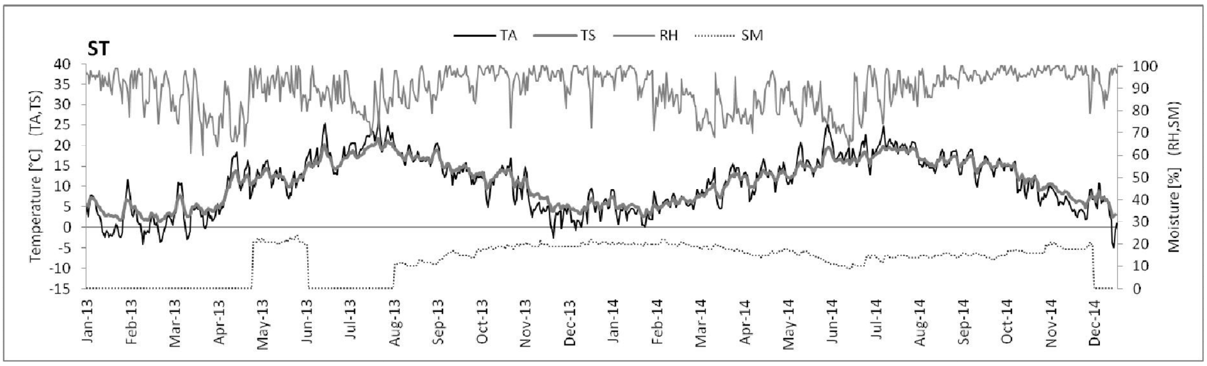


Staffort (ST)

Oberrheintiefland

122 m a.s.l.

Lon / Lat	8,5115 / 49,0763
Forest type	deciduous forest
Canopy density	small
Herb layer density	high
Herb layer coverage	continuous
Litter depth	small
Litter coverage	fragmentary
Soil / humus type	cambisol (WRB), mull-like moder
Comments	Moist forest with a very loose stand density. Moisture indicators are common (f.e. ash, aspen), a stream flows near the ground surface. Dense herbal- and grass-rich ground vegetation. Plenty of earth worm feces as well as mounds of burrowing animals. Small humic A-horizon. Small forested area, surrounded by agricultural land and roads.

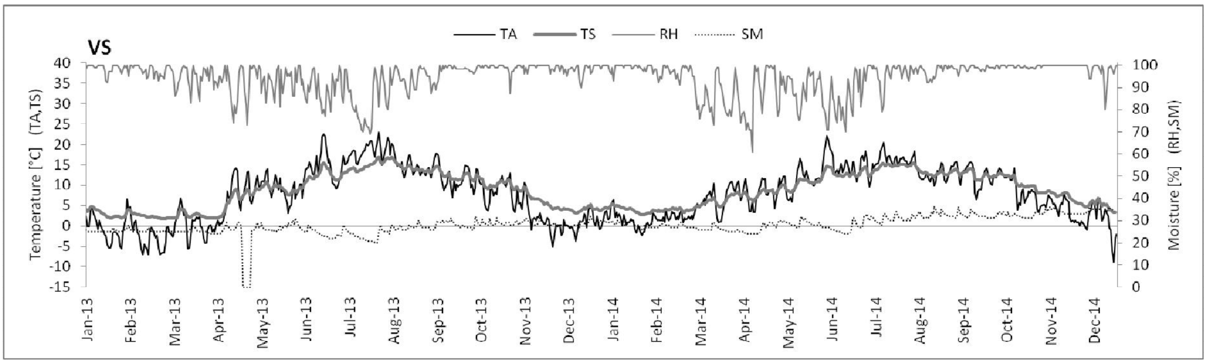


Villingen-Schwenningen (VS)

Schwäbisches K.-L.-Land / N.-T.-Gäuplatten

700 m a.s.l.

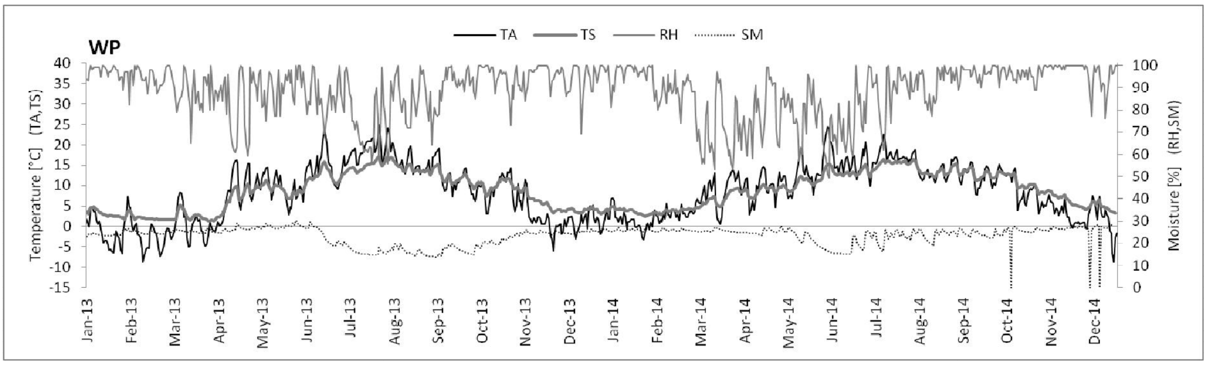
Lon / Lat	8,5666 / 48,0757
Forest type	coniferous forest
Canopy density	medium to high
Herb layer density	small to high
Herb layer coverage	fragmentary
Litter depth	small
Litter coverage	fragmentary; substitute: moss
Soil / humus type	rendzina, moder
Comments	Spruce timberland, with a high stand density. Young beech, spruce and white fir trees are common in the understorey. Ground cover is mainly made up by moss, herbs or grass (on forest paths). Located at the so called "Baar"- a landscape with a specific regional climate due to its topography (subalpine trough). Characterized by frequent frost occurrences and long-lasting lakes of cold air.



Wippingen (WP)

Schwäbische Alb 659 m a.s.l.

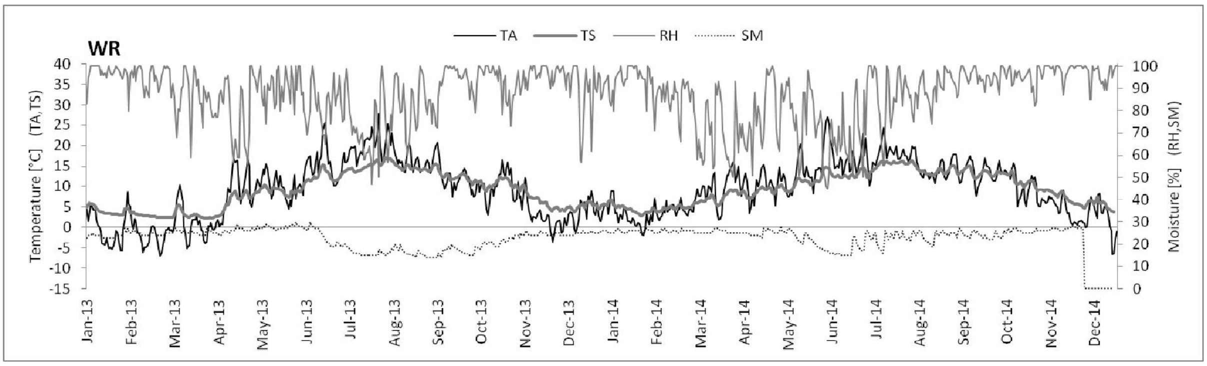
Lon / Lat	9,8400 / 48,4153
Forest type	deciduous forest
Canopy density	medium to high
Herb layer density	medium to small
Herb layer coverage	fragmentary
Litter depth	high
Litter coverage	continuous
Soil / humus type	rendzina; moder to raw humus
Comments	Beech timberland with a high stand density, in the middle of a larger forested area. The upper soil is very humic and heavily infused by fine roots. Plenty earth worm feces and a high variety on fungi has been found in autumn. A shooting stand was close to the investigated area.



Wüstenrot (WR)

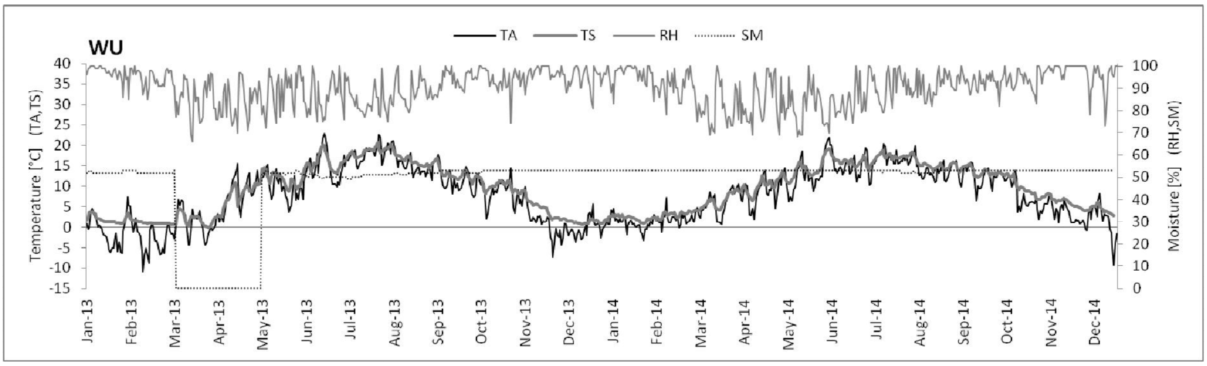
Schwäbisches Keuper-Lias-Land 504 m a.s.l.

Lon / Lat	9,4344 / 49,0994
Forest type	coniferous forest
Canopy density	medium to high
Herb layer density	small (high)
Herb layer coverage	continuous, patches of high coverage
Litter depth	medium to small
Litter coverage	continuous
Soil / humus type	Hangpseudogley; raw humus
Comments	Dense spruce timberland with single, adult beech trees located at a slope with northern sun exposition. High Herb layer cover occurs at clearances. A meadow borders at the north, a beech timberland at the west. Periodic spring water and moisture indicators (*Alnus glutinosa*) were found.



Wurzacher Ried (WU) *Alpenvorland* 665 m a.s.l.

Lon / Lat	9,9253 / 47,9390
Forest type	swamp
Canopy density	medium to small
Herb layer density	medium
Herb layer coverage	fragmentary
Litter depth	low
Litter coverage	fragmentary
Soil / humus type	histosol (WRB), raw humus
Comments	The area of investigation is a downy birch forest located at the north-eastern border of the nature protection area “Wurzacher Ried”, a large area of high and low moor biotopes. Parts of the area are covered by dense spruce stands. The forest ground is highly covered by moss. Distinct signs for beaver occurrence were found.



APPENDIX B

Tables and Figures corresponding to the chapter

MATERIALS AND METHODS

Meteorological station – Basic Site

-

HOBO Micro Station Data Logger H21-002, by Onset

Log interval: 10 sec

	abbr.	physical parameter	No.	unit	location	type	producer	sensor	measurement principle	resolution	measurement range	accuracy	drift
1	**Ts**	soil temperature	1	C°	5 cm ground depth	Temperature Smart Sensor S-TMB-M002	Onset		NA	< ±0.03°C (0° to 50°C)	-40° to 100°C	±0.2°C from 0° to 50°C	< ±0.1°C (0.18°F) per year
2	**Ta**	air temperature	1	C°	50 cm above ground	Temperature & RH Smart Sensor S-THB-M002	Onset	Sensirion SHT15	semi conductor	0.02°C at 25°C	-40°C to 75°C	+/- 0.21°C from 0° to 50°C	< 0.1°C (0.18°F) per year
3	**RH**	relative humidity		%	50 cm above ground			Sensirion SHT16	capacitive	0.1% RH at 25°C	0-100% RH at -40° to 75°C	+/- 2.5% from 10% to 90% RH (typical), to a maximum of +/- 3.5%	< 1% per year typical; hysteresis 1%
4	**MS**	soil moisture	1	m^3/m^3	5 cm ground depth	EC-5 Soil Moisture Smart Sensor - S-SMC-M005	Onset	Decagon ECH2O EC-5		0.0007 m^3/m^3 (0.07%)	0 to 0.550 (m^3/m^3)	±0.031 m^3/m^3 (±3.1%) typical 0 to 50°C ; ±0.020 m^3/m^3 (±2%) with soil specific calibration.	
	technical accessories		1		solar radiation shield RS2, Onset								

Data source: http://www.onsetcomp.com, http://www.rotronic.com, http://www.upgmbh.com, http://www.sensirion.com, http://www.stevenswater.com, http://www.vaisala.com, http://www.skyeinstruments.info

Meteorological station - Intensive Site

Logger: SDI-LOG40, by UP
Log interval: 10 sec

	abbr.	physical parameter	No.	unit	location	type	producer	sensor	measurement principle	resolution	measurement range	accuracy	drift
1	**T_S**	soil temperature	3	C°	5 cm ground depth	SKTS 200	Skye	10 k thermis-tor	NA	< 0,01 °C	0 to 60°C	0,2 °C	-
2	**T_A**	air temperature	3	C°	50 cm above ground	HygroClip2 (HC2)	Rotronic	PT100 1/3 DIN Class B	NA	-	-40°C to 85 °C	±0.2 K (at 23±5°C)	-
3	**RH_A**	relative humidity		%				HYGROMER® IN-1	NA	-	0 to 100%	±0.8 %rh (at 23±5°C)	<1 %rh / year
4	**T_I**	air temperature	1	C°	2 m above gr., inside the forest	HygroClip2 (HC2)	Rotronic	PT100 1/3 DIN Class B	NA	-	-40°C to 85 °C	±0.2 K (at 23±5°C)	-
5	**RH_I**	relative humidity		%				HYGROMER® IN-1	NA	-	0 to 100%	±0.8 %rh (at 23±5°C)	<1 %rh / year
6	**SM**	soil moisture	1	% ; S/m	5cm ground depth	Hydra Probe II Soil Moisture and Salinity Sensor	Stevens		NA		From completly dry to fully saturated, 0 to +55°C	± 0.01 WFV for most soils, ± 0.03 max for fine textured soils	
7	**T_O**	air temperature	1	°C	2 m above ground, outside the forest	Vaisala Weather Transmitter WXT520	Vaisala	THERMO CAP	capacitive ceramic	0.1 °C	-52°C to +60 °C	±0.3 °C at 20°C	
8	**RH_O**	relative humidity		%				HUMICAP	capacitive thin film polymer	0.1 %RH	0 ... 100 %RH	±3 %RH within 0 ... 90 %RH, ±5 %RH within 90 ... 100 %RH	
9	**AP**	barometric pressure		hPa				BAROCAP	capacitive silicon	0.1 hPa	600 ... 1100 hPa	±0.5 hPa at 0 ... +30 °C, ±1 hPa at -52 ... +60 °C	
10	**wind**	wind speed and direction		m/s, °					Ultrasonic 2D	0.1 m/s; 1°	0 ... 60 m/s; 0 ... 360°	±3% at 10m/s; ±3°	

11	**prec**	precipitation	mm, s				raindrop impact on steel cover with piezoelectrical sensor	0.1 mm/h	0 ... 200 mm/h (rain)	-	
12	**GR**	global radiation	W/m²	silicon cell pyranometer sensor SKS 1110	Skye	silicon photocell	NA	1 mV / 100 W/m²	0 - 5000 W/m² (350 - 1100 nm, -30 to +75°C, 0-100% RH)	Linearity err. - to above level < 0,2%, abs. cal. Err. typ. <3 %, cos-err. 3 %, azimuth err. <1 %	±2%
	technical accessories	1	Levelling unit for light sensors SKM 221		Skye						

Meteorological station - Intensive Site – part 2

B2 - VEGETATION SURVEY: FIELD CHECK LIST

Vegetation					
Standort:		Datum:		Uhrzeit:	

Waldbestand nach Kartierschlüssel LUBW:
Wetter: Vollsonne, zeitweise bewölkt, voll bewölkt, regnerisch, neblig

Stockwerke des Bestandes	Deckungsgrad Vegetation / Homogenität
1) Oberste Kronenschicht (Altbäume); > 20m	1)
2) Jungbäume, hohe Sträucher; ~ 5 – 20 m	2)
3) Sträucher, kleine Jungbäume; 1 – 5m	3)
4) Krautschicht (Gras, Stauden, Kraut, Jungbäume..); 0,05 – 1m	4)
5) Streuschicht (Streu, Moos) wenn homogen und mind. mitteldick	5)

STREUSCHICHT und BODEN					
Streu besteht aus:	*Nadeln, Zapfen, Buche, Eiche*				
Streu-Mächtigkeit:	*kaum*	*Gering (-2cm)*	*Mittel (2 – 5cm)*	*Hoch (>5cm)*	
Laub-Verteilung:	*gleichmäßig*	*Mosaikartig*	*→ Deckung ca….. %*		
Laub-Färbung:	*dunkel*	*mittel*	*hell*		
Wenn freie Flächen:	*Boden: dunkel / mittel / hell*	*Steine/Felsen, viel/mittel/wenig, frei/Moos*			
Humus und Wurzeln	*Oberflächl. dicker Wurzelfilz*	*Moder (viel unzersetzt) / Mull (gut zersetzt)*			
Anmerkungen:					

BAUMSCHICHT		
1) Arten Kronenschicht (D = Dominant)	2) Arten Jungbäume	*Anmerkungen*

Beschattung :	*Kaum*	*Gering*	*Mittel*	*Hoch (Nadelb.)*
Sonstiges:	*Alle ähnlich alt / dick*	*Forst erkennbar*	*Tote Bäume*	*Dicke Altbäume*

Bewirtschaftung erkennbar:	Frisch geschl. Baumstämme, Baumstümpfe alt/jung, Fahrrinnen, Schutzmaßn.
Epiphyten (Moos, Efeu):	Moos: Totholz / Steine / Baumbasis /z.T. bis Baumkrone / Boden Polster o. Flächig
Totholz im Bestand:	Stämme, Äste groß / klein, lose verteilt, Haufen, viel / mittel / wenig

STRAUCHSCHICHT	
Straucharten , Auftreten einzeln (E)/ Gruppen (G);	*Anmerkungen (Fraßschäden?)*

KRAUTSCHICHT (Frühlings-Geophyten)		
Wichtige Arten und DG	Gräser am Standort	Feuchte-/ Schattenzeiger am Standort
		Nasse Stellen (GW, Stauwasser)

Eindruck Homogenität (Laub, Kraut, Strauch)	*(Mosaikartige Verteilung der Arten, Mulden/ Erhöhungen mit anderen Arten,..)*

AM	Artname	B	S	PZ	AM	Artname	B	S	PZ

Artenmächtigkeit AM: D – Dominant ; U – Untergeordnet
Soziabilität S: 1 – einzeln wachsend; 2 – Gruppen/Horstweise wachsend
3 –Polster /Flecken; 4 –viele Gruppen, kleinflächig; 5 – großflächig
Phänologischer Zustand PZ: k = Keimplanze, j = Jungpflanze, kn = knospend, b = blühend
F = fruchtend, a = austreibend (bei mehrjährigen), v = absterbend
E = entlaubt, t = tot / vertrocknet, VB = verblüht
Beschattung B: Dominante Art beschattet den Boden 1: kaum, 2: mittel , 3: stark

Auch wenn die Laubschicht im Frühjahr durch Geophyten durchbrochen ist, gilt es den Deckungsgrad insgesamt abzuschätzen (mit den von Geophyten bestandenen Flächen); außer dies ist im Herbst schon geschehen, dann kann dies hier entfallen (Flächenhaft Schutz vorhanden oder nicht?)

Baumschicht - Beschattung im Frühling entsteht einmal durch die Baumstämme selbst oder durch erste Belaubung oder durch immergrüne Bäume

→ *wenig und dünne Bäume: kaum; viele und auch dicke Bäume / Belaubung bei lockerem Bestand schon eingesetzt: gering; Belaubung hat schon zum Teil eingesetzt bei hoher Baumdichte/ lichter Nadelwald: mittel; dichter Nadelwald: hoch*

B3 - TICK SAMPLING (FLAGGING): CHECK LIST

study site	date	time	wind (1= calm, 2 = light, 3 = strong)	soil (dry or moist)	litter and humus (dry or moist)

drag	nymphs	male	female	plant height	sunny, shady or mixed
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					

Plant height		**number of drags**
High = H	> 30 cm (shrubs, bushes, long grass..)	2 times
Medium = M	10 – 30 cm (short grass, herbage..)	4 times
Low = L	0 – 10 cm (herbage, moss, litter, soil)	4 times

Total number of larvae

B4 - TICK DATA (NYMPHS) : ORIGINAL (A) AND CORRECTED (B) 2013

A

2013	AH	AL	AW	BT	CW	DS	EP	FB	FN	FR	GH	HQ	HW	KT	NA	MB	PH	PK	RF	ST	SW	VS	WP	WR	WU
Jan	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Feb	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Mar	0	14	7	52	0	NA	6	NA	NA	22	NA	NA	24	NA	37	0	0	1	NA	1	NA	NA	NA	1	NA
Apr	1	81	69	190	25	13	47	0	146	54	0	40	74	31	32	30	1	13	3	198	2	0	21	16	NA
May	1	68	86	276	NA	NA	NA	0	NA	38	NA	46	40	NA	NA	77	8	24	NA	73	16	NA	NA	NA	NA
Jun	0	46	27	NA	30	13	52	0	154	94	14	96	62	0	24	43	17	25	42	116	9	7	45	21	NA
Jul	1	26	8	109	34	11	6	0	15	28	10	67	47	24	9	61	7	20	16	200	16	0	13	8	0
Aug	0	13	13	160	14	4	5	0	34	11	5	53	31	6	1	13	9	12	22	50	14	1	35	14	1
Sep	0	9	12	42	5	2	4	0	15	6	3	30	12	8	3	7	9	4	7	29	12	0	18	11	0
Oct	0	6	4	28	2	4	2	0	5	11	0	9	7	3	1	28	2	2	4	22	1	0	8	8	0
Nov	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Dec	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

B

2013	AH	AL	AW	BT	CW	DS	EP	FB	FN	FR	GH	HQ	HW	KT	MB	NA	PH	PK	RF	ST	SW	VS	WP	WR	WU
Jan	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Feb	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Mar	0	14	7	52	0	NA	6	0	14	22	1	10	24	3	0	37	0	1	4	1	2	0	5	1	0
Apr	1	81	69	190	25	13	47	0	146	54	0	40	74	31	30	32	1	13	3	198	2	0	21	16	0
May	1	68	86	276	32	14	36	0	113	38	10	46	40	30	77	31	8	24	29	73	16	2	43	23	0
Jun	0	46	27	209	30	13	52	0	154	94	14	96	62	26	43	24	17	25	42	116	9	7	45	21	0
Jul	1	26	8	109	34	11	6	0	15	28	10	67	47	24	61	9	7	20	16	200	16	0	13	8	0
Aug	0	13	13	160	14	4	5	0	34	11	5	53	31	6	13	1	9	12	22	50	14	1	35	14	1
Sep	0	9	12	42	5	2	4	0	15	6	3	30	12	8	7	3	9	4	7	29	12	0	18	11	0
Oct	0	6	4	28	2	4	2	0	5	11	0	9	7	3	28	1	2	2	4	22	1	0	8	8	0
Nov	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Dec	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

B4 - TICK DATA (NYMPHS) : ORIGINAL (A) AND CORRECTED (B) 2014

A

2014	AH	AL	AW	BT	CW	DS	EP	FB	FN	FR	GH	HQ	HW	KT	MB	NA	PH	PK	RF	ST	SW	VS	WP	WR	WU
Jan	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Feb	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Mar	0	74	48	NA	NA	NA	NA	0	NA	35	NA	9	5	NA	31	NA	1	2	NA	24	12	NA	NA	NA	NA
Apr	0	114	21	118	30	18	9	0	42	23	1	48	70	2	28	24	4	3	35	26	2	19	6	30	7
May	1	144	57	6	8	NA	0	0	50	20	13	72	71	2	42	0	4	11	8	87	85	8	0	0	1
Jun	2	26	14	12	5	14	6	0	17	9	9	36	33	1	26	18	4	13	4	11	14	5	0	2	14
Jul	0	7	6	8	7	5	15	0	13	8	2	37	29	1	16	12	7	5	0	13	8	2	3	5	1
Aug	0	0	1	0	5	7	8	0	9	2	0	18	11	0	6	9	1	3	2	13	30	3	7	2	4
Sep	0	2	6	3	4	2	0	0	21	9	0	18	5	0	10	0	2	5	8	7	11	0	1	1	9
Oct	1	7	10	0	0	4	0	0	18	15	0	7	6	1	8	0	2	4	6	6	4	0	2	0	3
Nov	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Dec	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

B

2014	AH	AL	AW	BT	CW	DS	EP	FB	FN	FR	GH	HQ	HW	KT	MB	NA	PH	PK	RF	ST	SW	VS	WP	WR	WU
Jan	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Feb	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Mar	0	74	48	22	9	11	6	0	26	35	4	9	5	1	31	10	1	2	10	24	12	6	3	6	6
Apr	0	114	21	118	30	18	9	0	42	23	1	48	70	2	28	24	4	3	35	26	2	19	6	30	7
May	1	144	57	6	8	21	0	0	50	20	13	72	71	2	42	0	4	11	8	87	85	8	0	0	1
Jun	2	26	14	12	5	14	6	0	17	9	9	36	33	1	26	18	4	13	4	11	14	5	0	2	14
Jul	0	7	6	8	7	5	15	0	13	8	2	37	29	1	16	12	7	5	0	13	8	2	3	5	1
Aug	0	0	1	0	5	7	8	0	9	2	0	18	11	0	6	9	1	3	2	13	30	3	7	2	4
Sep	0	2	6	3	4	2	0	0	21	9	0	18	5	0	10	0	2	5	8	7	11	0	1	1	9
Oct	1	7	10	0	0	4	0	0	18	15	0	7	6	1	8	0	2	4	6	6	4	0	2	0	3
Nov	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Dec	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

APPENDIX C

Tables and Figures corresponding to the chapter

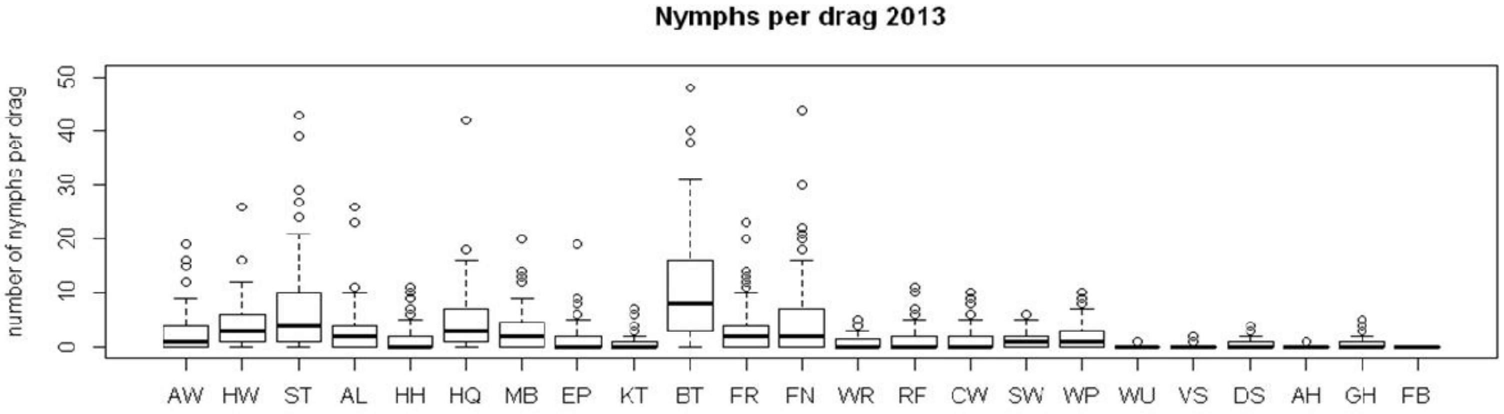
RESULTS AND DISCUSSION

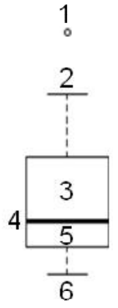
C1 - TICK DATA : LARVAE PER DRAG IN 2013 AND 2014

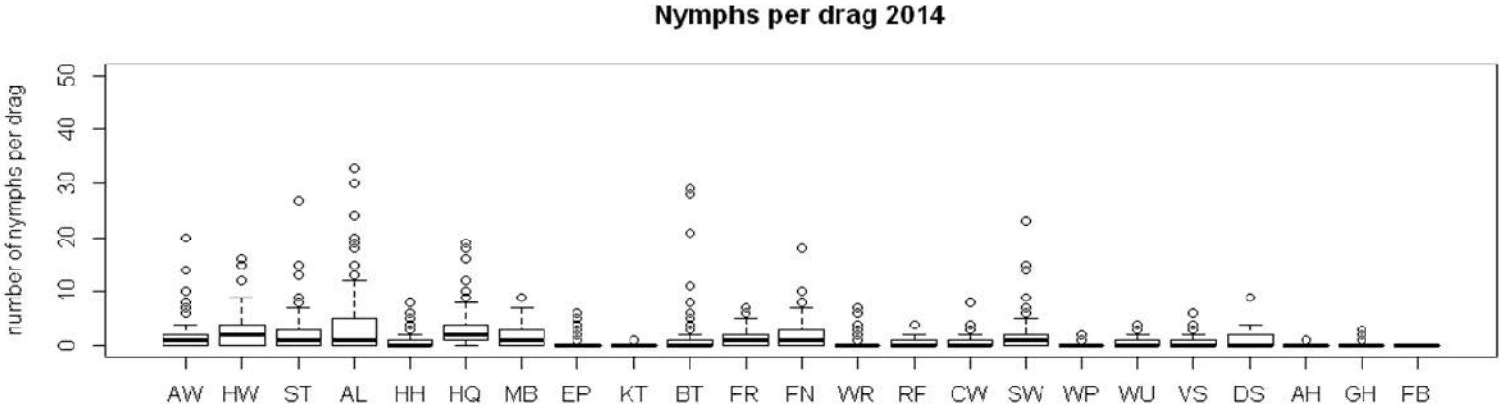
year	month	**AH**	**AL**	**AW**	**BT**	**CW**	**DS**	**EP**	**FB**	**FN**	**FR**	**GH**	**HQ**	**HW**	**KT**	**MB**	**NA**	**RF**	**ST**	**SW**	**VS**	**WP**	**WR**	**WU**
2013	3	0	0	0	0	0	NA	0	NA	NA	0	NA	NA	0	NA	0	0	NA	0	NA	NA	NA	0	NA
2013	4	0	0	0	26	30	0	1	NA	0	0	0	29	0	36	0	31	0	0	0	0	0	21	NA
2013	5	0	11	0	6000	0	0	0	0	0	0	0	0	0	0	35	0	0	10	0	0	0	0	NA
2013	6	0	0	0	NA	42	2	18	0	184	0	4	74	2	0	0	0	18	41	89	2	3	16	NA
2013	7	0	40	1	2400	218	1	0	0	0	0	1	608	18	1	13	2	0	48	21	0	9	0	0
2013	8	0	2	1	517	5	0	63	0	97	406	0	38	70	7	0	1	18	18	0	2	7	12	0
2013	9	0	1	48	63	0	0	0	0	17	5	0	7	2	0	51	0	0	12	55	0	0	0	0
2013	10	0	0	4	0	0	0	0	0	0	7	0	7	44	0	91	0	0	7	0	0	0	0	0
larvae 2013		0	54	54	9006	295	3	82	0	298	418	5	763	136	44	190	34	36	136	165	4	19	49	0
2014	3	0	0	0	NA	NA	NA	NA	NA	NA	0	NA	0	0	NA	0	NA	NA	0	0	NA	NA	NA	NA
2014	4	0	1	0	178	14	0	0	0	37	0	0	3	30	0	0	0	0	6	0	0	0	0	0
2014	5	0	0	11	0	0	NA	0	0	94	0	0	60	22	0	11	0	0	253	1	1	0	0	0
2014	6	0	4	10	0	0	NA	3	0	21	195	0	10	2	0	0	4	12	211	0	0	0	0	7
2014	7	0	33	0	6	0	25	0	0	39	85	0	8	8	0	0	1	0	5	0	3	1	0	0
2014	8	0	0	8	17	0	0	1	0	322	273	NA	64	207	0	11	0	0	60	67	0	5	0	1
2014	9	0	0	0	27	1	0	0	0	109	7	NA	23	0	0	0	0	5	12	2	NA	0	8	1
2014	10	0	8	0	0	0	1	0	0	15	19	NA	0	5	3	0	0	4	12	0	0	2	0	0
larvae 2014		0	46	29	228	15	26	4	0	637	579	0	168	274	3	22	5	21	559	70	4	8	8	9

year	month	**AH**	**AL**	**AW**	**BT**	**CW**	**DS**	**EP**	**FB**	**FN**	**FR**	**GH**	**HQ**	**HW**	**KT**	**MB**	**NA**	**RF**	**ST**	**SW**	**VS**	**WP**	**WR**	**WU**
2013	3	0	1	3	5	0	NA	0	NA	NA	4	NA	NA	1	NA	0	2	NA	0	NA	NA	NA	0	NA
2013	4	0	5	3	8	5	0	1	NA	6	1	0	3	5	1	2	5	0	3	1	0	0	2	NA
2013	5	0	8	5	26	0	0	0	0	0	16	0	1	1	0	7	0	0	1	1	0	0	0	NA
2013	6	0	9	7	NA	5	0	3	0	3	3	3	8	7	0	6	13	4	3	2	4	1	1	NA
2013	7	0	11	2	9	5	0	0	0	2	18	4	12	1	0	3	3	1	2	1	0	0	1	2
2013	8	0	1	4	5	2	0	0	0	1	2	1	6	0	1	0	4	0	2	1	2	0	0	0
2013	9	0	3	3	7	2	0	0	0	0	3	0	7	3	2	1	1	0	3	2	0	0	0	0
2013	10	0	2	1	5	0	0	0	0	0	2	0	3	0	0	8	0	0	8	1	0	0	0	0
adults 2013		0	40	28	65	19	0	4	0	12	49	8	40	18	4	27	28	5	22	9	6	1	4	2
2014	3	0	13	6	NA	NA	NA	NA	NA	NA	12	NA	5	3	NA	7	NA	NA	11	3	NA	NA	NA	NA
2014	4	0	11	0	19	3	2	1	0	2	87	12	8	3	5	2	7	20	11	1	7	4	1	0
2014	5	1	5	14	3	1	NA	0	0	15	49	4	8	13	4	7	0	2	8	11	0	1	0	1
2014	6	0	23	2	0	4	NA	0	0	3	1	0	6	2	0	2	12	6	2	3	7	0	2	0
2014	7	0	3	0	3	2	1	4	0	0	7	3	2	5	0	4	5	1	1	1	3	0	1	0
2014	8	0	2	3	4	4	2	2	0	0	1	NA	3	2	1	0	0	1	2	7	4	0	1	0
2014	9	0	0	0	1	1	2	1	0	1	0	NA	0	0	0	0	0	1	1	12	NA	0	1	0
2014	10	0	0	0	0	0	0	0	0	2	0	NA	0	0	0	0	0	0	2	0	4	0	0	2
adults 2014		1	57	25	30	15	7	8	0	23	157	19	32	28	10	22	24	31	38	38	25	5	6	3

C2 – TICK DATA: NYMPHS PER DRAG IN 2013 AND 2014



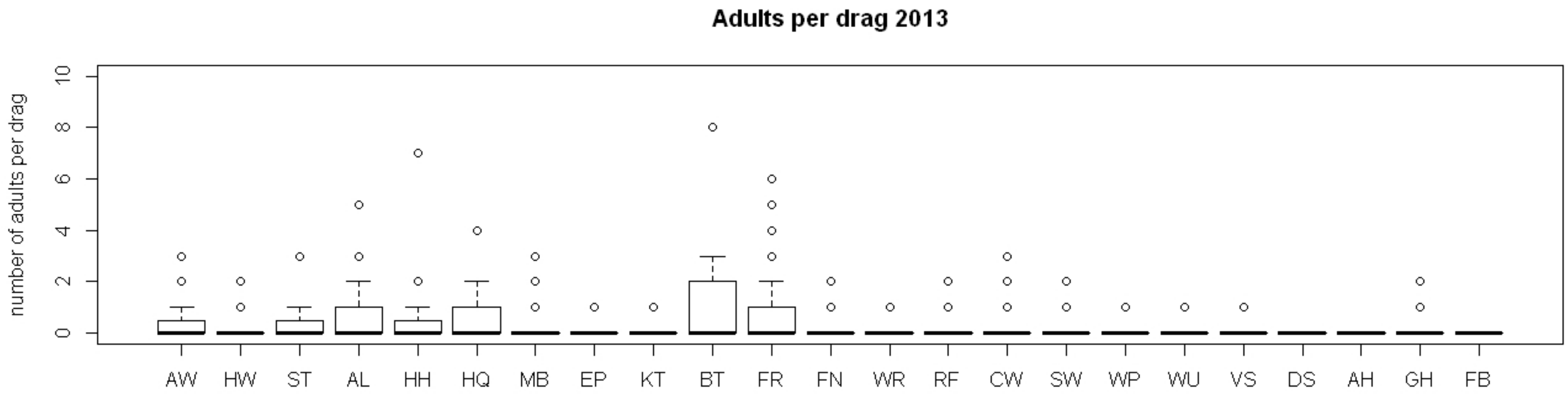


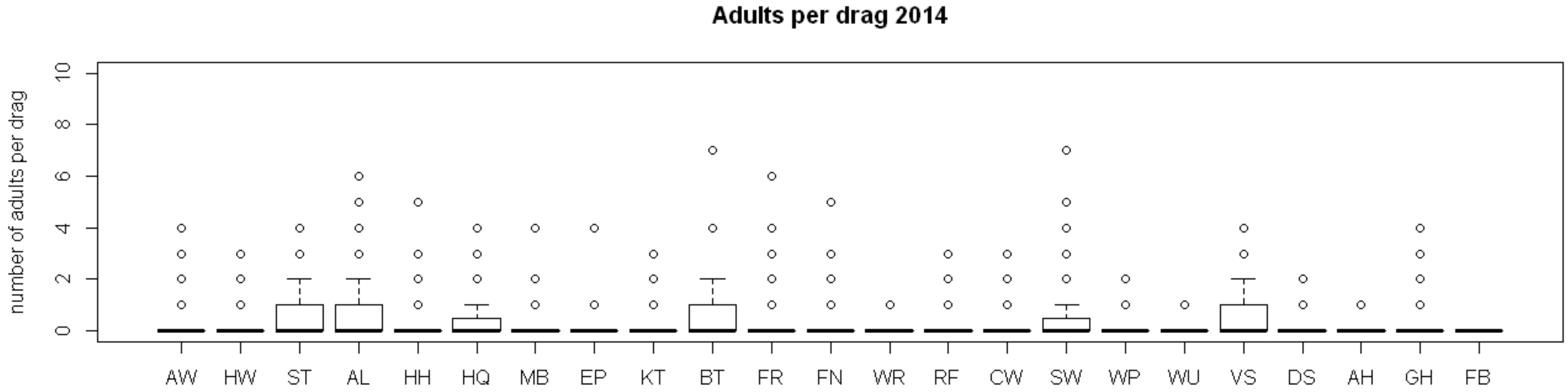


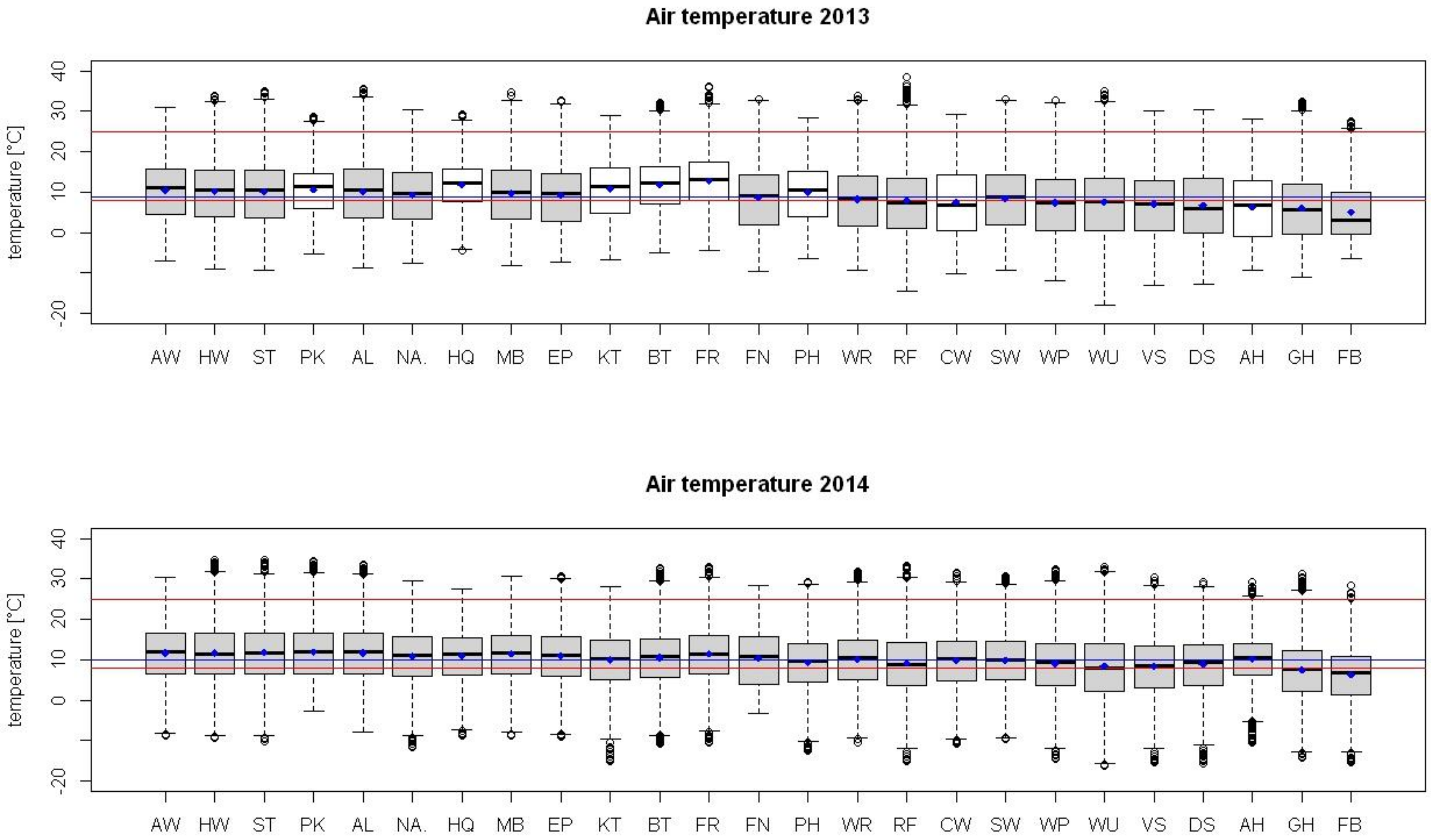
1-outlier
2-max
3-upper quartile
4-median
5-lower quartile
6-min

The legend is representative for all boxplots illustrated in the appendix.

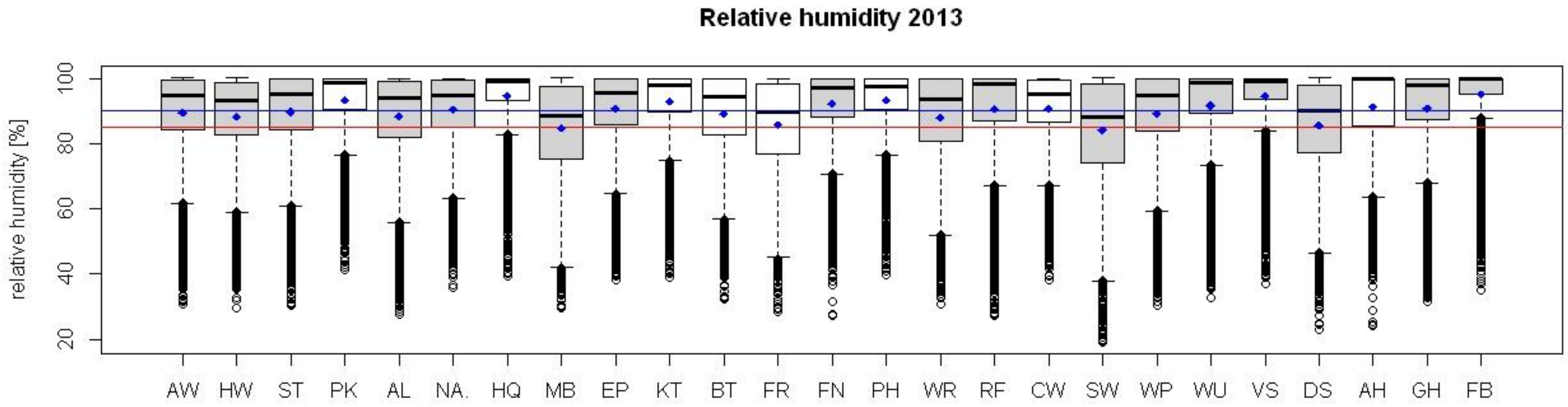
C2 – TICK DATA: ADULTS PER DRAG IN 2013 AND 2014

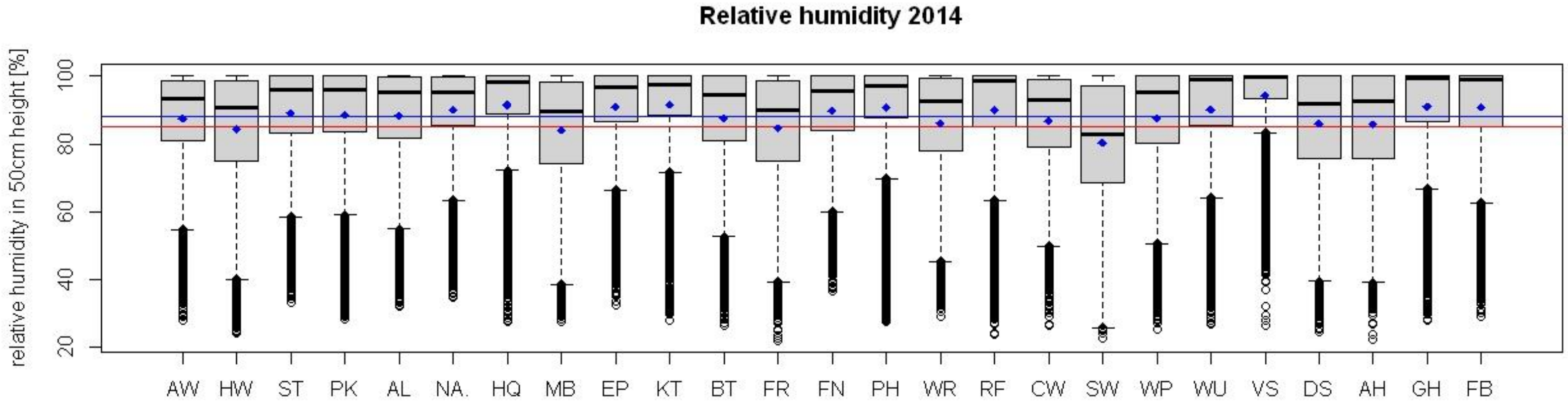




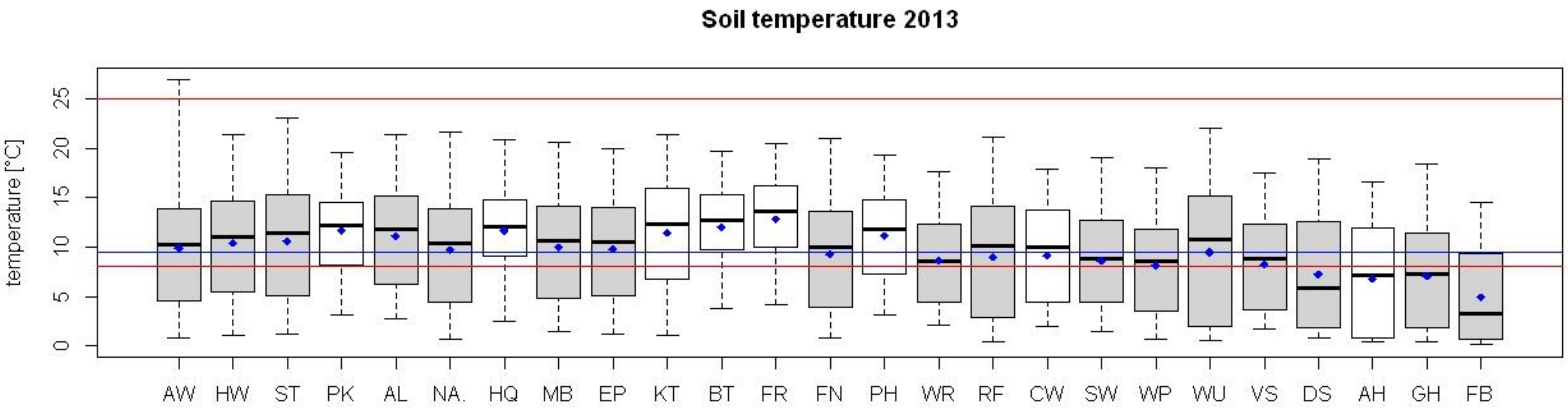


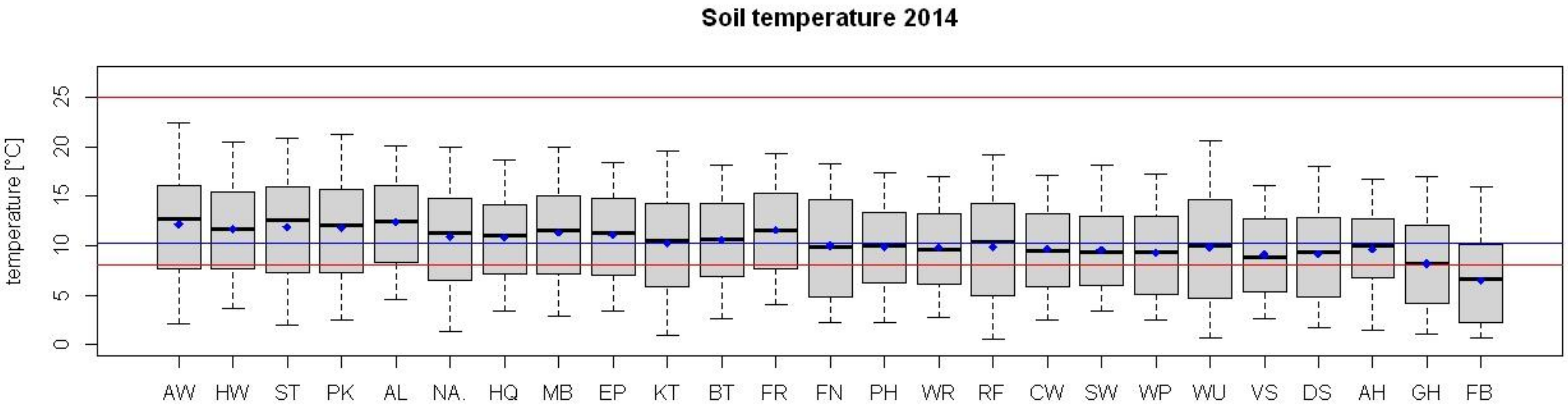
Red lines depict the maximum and minimum of the ticks' physiological optimum range. Blue dots/line represent the mean of the data.



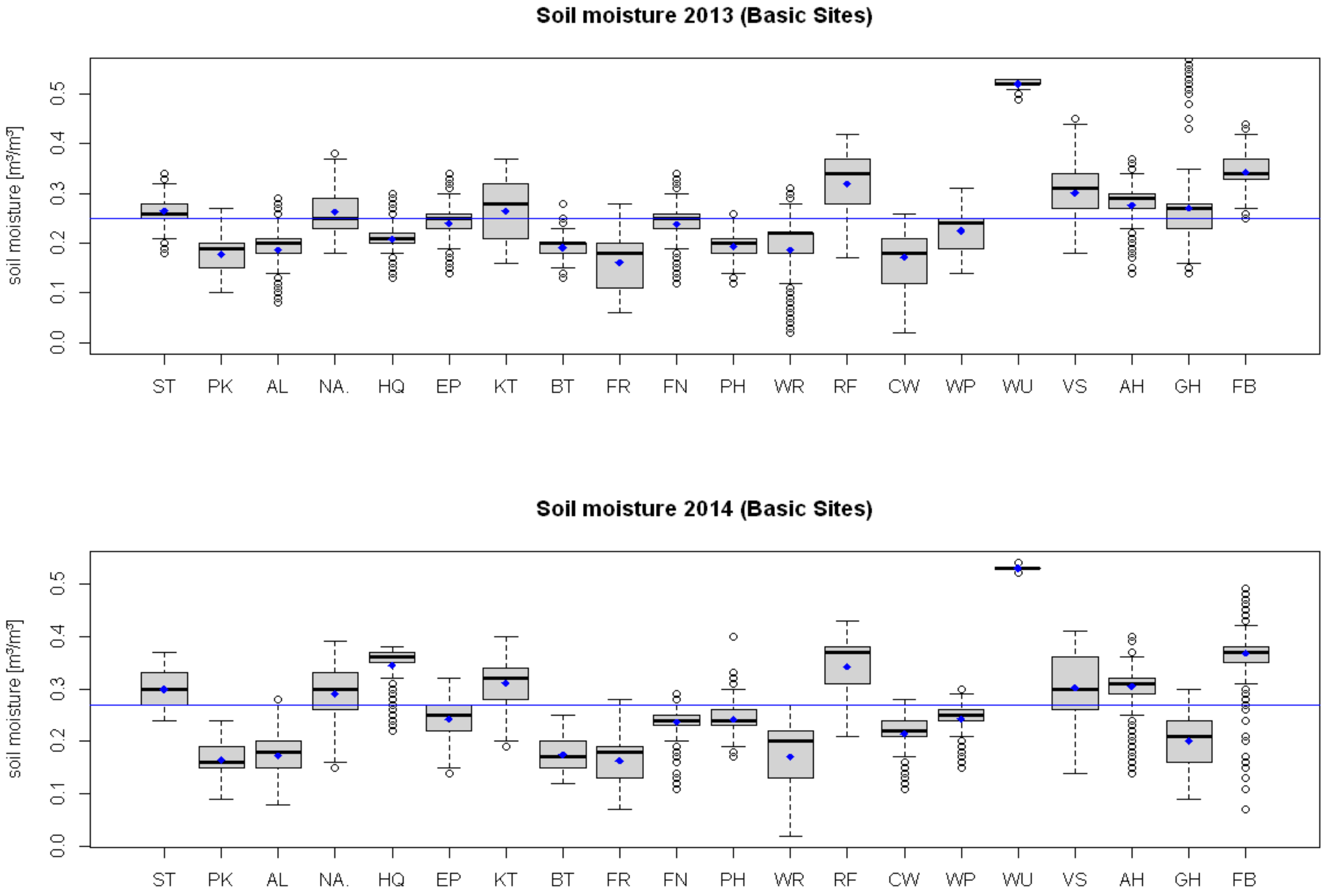


The red line depict the equilibrium humidity of 85% for *Ixodes ricinus*. Blue dots/line represent the mean of the data.

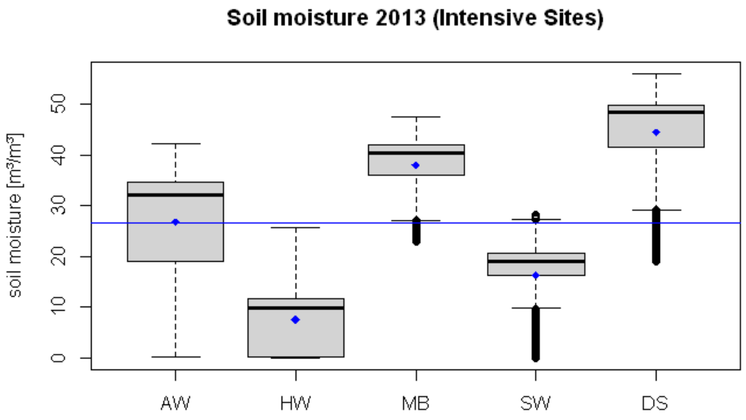


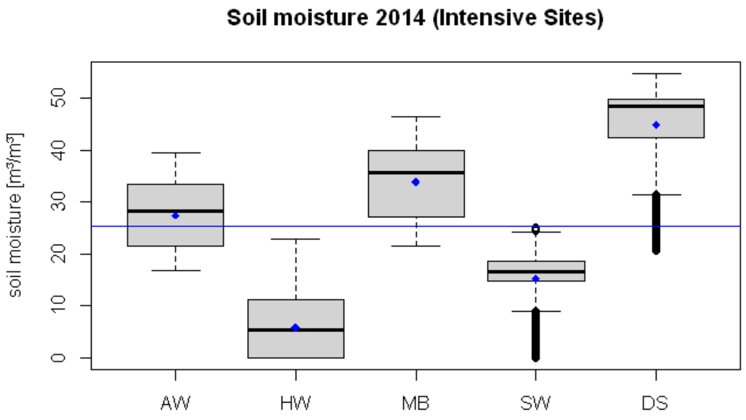


Red lines depict the maximum and minimum of the ticks’ physiological optimum range. Blue dots/line represent the mean of the data.



Blue dots/line represent the mean of the data. Data basis: 10 minute averages.





Blue dots/line represent the mean of the data. Data basis: 10 minute averages.

			Soil temperature [°C]					Air temperature [°C]						Relative humidity [%]					
	Site	Statistics	**T_S1**	**T_S2**	**T_S3**	**T_S4**	**SM**	**T_L**	**T_A1**	**T_A2**	**T_AIn**	**T_AOut**	**T_ADWD**	**RH_L**	**RH_A1**	**RH_A2**	**RH_AIn**	**RH_AOut**	**RH_{DWD}**
2013	**AW**	Min	1.6	1.8	1.6	1.2	17	0.1	-7.2	-6.9	-6.9	-7.1	-7.6	61	31	31	29	29	21
		1st Qu.	4.5	4.2	4.5	5.0	27	10.7	4.3	4.4	4.4	4.1	3.8	100	82	84	77	72	71
		Median	8.2	6.4	9.6	11.6	33	13.8	11.1	11.2	11.1	10.7	10.6	100	93	94	90	86	85
		Mean	9.0	7.4	9.8	10.6	30	13.8	10.3	10.6	10.5	10.3	10.5	99	89	89	85	81	81
		3rd Qu.	12.7	11.2	14.1	15.1	35	17.3	15.7	15.9	15.9	15.5	16.1	100	99	100	98	93	94
		Max	21.4	15.3	27.0	21.9	42	29.3	31.2	31.6	33.9	34.1	37.6	100	100	100	100	97	100
	HW	Min	3.6	2.0	2.3	2.2	0	0.6	-9.1	-9.1	-8.9	-9.4	-7.6	47	24	24	23	20	21
		1st Qu.	5.6	4.5	5.2	5.8	0	9.2	3.7	3.7	3.8	3.6	3.8	99	77	76	74	69	71
		Median	7.0	6.7	10.3	10.9	6	13.2	10.3	10.3	10.4	10.1	10.6	100	91	90	89	83	85
		Mean	7.8	7.8	10.3	10.4	6	13.2	10.2	10.2	10.3	10.2	10.5	97	85	85	83	78	81
		3rd Qu.	10.1	11.1	15.4	14.4	11	16.9	15.5	15.5	15.6	15.4	16.1	100	99	98	97	91	94
		Max	15.9	19.6	21.4	20.5	26	32.2	36.9	36.8	37.1	38.1	37.6	100	100	100	100	95	100
	MB	Min	3.1	1.8	1.6	1.1	18	0.7	-7.9	-8.2	-7.8	-7.9	-7.6	59	29	29	27	22	21
		1st Qu.	4.8	4.1	4.8	4.7	36	9.0	3.3	3.2	3.4	3.5	3.8	100	76	75	72	66	71
		Median	6.9	6.0	10.4	10.4	40	12.7	10.1	10.0	10.2	10.3	10.6	100	89	88	87	80	85
		Mean	7.8	7.0	9.9	9.7	38	12.6	9.8	9.7	9.9	10.1	10.5	98	85	84	83	76	81
		3rd Qu.	10.8	10.6	14.4	14.0	42	16.5	15.4	15.3	15.6	15.8	16.1	100	98	97	98	89	94
		Max	14.6	14.7	20.5	20.8	47	29.6	34.6	35.0	35.8	36.4	37.6	100	100	100	100	95	100
	SW	Min	1.1	1.1	1.8	1.5	0	-2.3	-9.1	-9.4	-8.9	-9.3	-12.9	36	19	19	16	18	16
		1st Qu.	2.4	4.3	3.9	3.9	16	8.8	1.8	1.9	1.8	1.7	0.4	100	75	76	71	66	73
		Median	5.9	8.9	7.4	8.9	19	13.4	8.8	8.8	8.9	8.7	7.1	100	89	89	85	80	91
		Mean	7.4	8.7	7.9	8.6	16	13.0	8.4	8.5	8.6	8.5	7.1	96	84	85	82	76	84
		3rd Qu.	11.3	12.8	11.7	12.7	21	17.4	14.3	14.3	14.5	14.3	13.1	100	99	99	97	90	97
		Max	19.8	19.1	18.1	19.6	28	34.2	33.1	33.0	33.3	33.5	32.5	100	100	100	100	97	100
	DS	Min	0.5	1.0	0.8	0.8	19	-4.1	-12.8	-12.9	-11.9	-12.9	-21.9	39	24	22	20	20	22
		1st Qu.	1.1	1.7	1.5	2.3	42	2.4	-0.1	-0.1	-0.1	-0.1	0.5	100	78	76	73	70	76
		Median	3.6	8.5	2.9	6.1	49	9.1	5.9	5.9	6.2	5.9	7.0	100	91	90	88	84	89
		Mean	5.2	8.0	4.1	7.5	44	8.9	6.7	6.7	6.9	6.8	7.0	97	86	85	83	79	83
		3rd Qu.	9.0	13.3	6.6	12.9	50	14.5	13.5	13.4	13.7	13.5	12.9	100	98	97	97	92	94
		Max	17.3	18.6	11.9	18.4	56	30.9	30.5	30.6	31.4	31.8	33.1	100	100	100	100	97	100

			Soil temperature [°C]					Air temperature [°C]						Relative humidity [%]					
	Site	Statistics	T_S1	T_S2	T_S3	T_S4	SM	T_L	T_A1	T_A2	T_AIn	T_AOut	T_ADWD	RH_L	RH_A1	RH_A2	RH_AIn	RH_AOut	RH_{DWD}
2014	**AW**	Min	1.8	2.6	1.8	2.4	17	-1.4	-8.7	-8.5	-8.4	-9.0	-12.0	42	29	27	25	25	20
		1st Qu.	7.7	7.7	7.7	7.3	22	6.8	6.5	6.7	6.8	6.3	6.8	96	81	81	76	70	68
		Median	12.8	12.6	12.6	12.2	28	12.4	11.8	12.1	11.9	11.4	12.0	99	93	93	91	85	84
		Mean	12.3	12.1	12.1	11.8	27	11.9	11.6	11.8	11.9	11.6	12.2	96	88	88	85	79	78
		3rd Qu.	16.2	16.0	15.9	15.7	33	16.6	16.5	16.7	16.7	16.3	17.3	100	99	98	99	92	93
		Max	23.0	22.1	22.2	21.1	40	28.0	30.9	31.5	32.5	34.6	36.5	100	100	100	100	95	100
	HW	Min	3.8	4.0	3.2	4.3	0	-1.0	-9.3	-9.3	-9.2	-9.7	-12.0	54	23	23	22	20	20
		1st Qu.	7.6	7.5	7.3	7.4	0	6.8	6.0	6.0	6.3	5.9	6.8	94	76	75	73	67	68
		Median	11.4	11.2	11.2	11.1	5	11.4	10.8	10.8	10.9	10.4	12.0	98	91	91	89	83	84
		Mean	11.4	11.2	11.2	11.1	6	11.3	11.1	11.1	11.2	11.0	12.2	96	85	84	83	77	78
		3rd Qu.	15.0	14.8	15.0	14.7	11	15.5	15.8	15.8	15.8	15.6	17.3	100	99	98	98	91	93
		Max	20.3	19.4	20.5	18.7	18	27.6	34.9	34.8	35.1	37.2	36.5	100	100	100	100	100	100
	MB	Min	2.3	3.4	3.1	2.9	22	-2.1	-8.6	-8.8	-8.1	-8.1	-12.0	47	27	27	27	22	20
		1st Qu.	6.9	7.5	7.2	6.8	27	6.5	6.4	6.3	6.6	6.6	6.8	100	75	74	72	63	68
		Median	11.4	11.5	11.6	11.2	36	11.7	11.8	11.7	11.9	12.0	12.0	100	90	89	88	79	84
		Mean	11.2	11.4	11.4	11.0	34	11.3	11.5	11.4	11.7	11.9	12.2	97	84	84	83	74	78
		3rd Qu.	15.0	15.0	15.2	14.9	40	15.7	16.2	16.1	16.4	16.6	17.3	100	99	98	100	87	93
		Max	20.1	19.8	20.0	18.9	46	28.3	30.7	30.7	31.8	33.7	36.5	100	100	100	100	93	100
	SW	Min	1.7	3.3	3.1	2.7	0	-0.1	-9.5	-9.8	-9.2	-3.6	-13.4	47	22	23	18	20	20
		1st Qu.	5.5	6.6	6.2	5.9	15	7.7	5.0	5.0	5.0	6.0	3.7	99	69	69	65	56	70
		Median	9.4	9.2	9.3	9.6	17	11.9	9.9	9.9	10.1	11.2	9.0	100	83	83	79	70	88
		Mean	9.5	9.7	9.4	9.5	15	11.6	9.9	10.0	10.0	11.1	8.8	96	80	80	77	68	81
		3rd Qu.	13.2	13.0	12.7	13.0	19	15.2	14.5	14.5	14.7	15.4	13.5	100	97	97	95	82	96
		Max	20.0	22.1	16.8	19.1	25	35.9	30.7	30.7	30.7	31.7	31.0	100	100	100	100	94	100
	DS	Min	1.1	2.4	1.4	2.0	21	-1.7	-15.4	-15.6	-14.8	-15.1	-22.9	34	25	24	25	24	20
		1st Qu.	4.6	6.6	4.5	4.9	42	3.9	3.8	3.7	3.9	3.7	3.1	100	77	75	71	66	73
		Median	9.4	10.3	9.5	9.2	49	9.6	9.3	9.2	9.4	9.1	8.6	100	93	91	89	82	90
		Mean	9.0	9.9	9.1	9.1	45	9.1	8.9	8.9	9.1	9.0	8.7	97	87	85	83	77	83
		3rd Qu.	12.8	13.1	12.8	12.8	50	13.6	13.7	13.6	13.8	13.7	13.8	100	100	100	99	90	97
		Max	17.7	18.2	18.1	16.5	55	29.0	29.1	29.4	30.2	31.4	31.6	100	100	100	100	95	100

C4 – MICROCLIMATE: NUMBER OF MISSING DATA (10 MINUTE AVERAGES)

			T_S1	T_S2	T_S3	T_S4	SM	T_L	T_A1	T_A2	T_AIn	T_AOut	T_ADWD	RH_L	RH_A1	RH_A2	RH_AIn	RH_AOut	RH_{DWD}
2013	AW	**NA's**	2628	3976	2345	475	475	2886	475	475	475	475	0	2886	475	475	475	475	0
	HW		5901	2891	649	0	1	2409	0	0	0	0	0	2409	0	0	0	0	0
	MB		6751	3129	338	0	0	2462	0	0	0	0	0	2410	0	0	0	0	0
	SW		1810	0	912	0	0	4528	0	0	0	0	0	1910	0	2497	0	0	0
	DS		2820	2703	3684	1394	1394	2749	1394	1394	1394	1394	0	2749	1394	1394	1394	1394	0
2014	AW	**NA's**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	HW		684	684	684	684	684	684	684	684	684	684	0	671	671	671	671	671	0
	MB		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	SW		969	969	969	984	984	3305	1111	1111	1111	2609	1	3022	1111	1111	1111	2609	1
	DS		0	1027	0	0	0	0	0	0	0	0	1	7	0	0	0	0	1

C5 – LIST OF PLANT SPECIES FOR EACH STUDY SITE

Oberrheintiefland					*transition area*		*Schwarzwald*			*Neckar-u. Tauber Gäuplatten*				*trans.a.*	*Schwäbisches Keuper-Lias-Land*				
AL	**AW**	**HW**	**PK**	**ST**	**HQ**	**FR**	**AH**	**FB**	**SW**	**CW**	**EP**	**MB**	**NA**	**VS**	**BT**	**KT**	**PH**	**RF**	**WR**
Q.ro	F.syl	**Pi.syl**	**Pi.syl**	Ac.ps	**F.syl**	**F.syl**	**F.syl**	**P.ab**	**F.syl**	**F.syl**	F.syl	**F.syl**	Fr.ex	**P.ab**	F.syl	F.syl	**F.syl**	-	**P.ab**
Fr.ex	Ac.sp	**F.syl**	**F.syl**	Fr.ex	S.sp	**Q.sp**	**P.ab**	F.syl	**A.al**	P.ab	Q.ro	Pr.av	S.sp	A.al	**Q.sp**	Q.ro	C.be	Pi.sp	F.syl
C.be	C.be	Q.ro	Q.sp	Po.tr	C.be	C.be	A.al	F.syl	Ac.ps	Pi.syl	C.be	Li.tu	S.al	Pi.syl	P.ab	C.be	Q.ro	Cr.sp	P.ab
Po.tr	Fr.ex	Q.pe	Ru.sp	Q.ro	Ac.sp	Ac.ps	**A.al**	Ac.ps	P.ab	Ru.sp	Ac.ps	C.be	Ac.ps	**F.syl**	F.syl	T.sp	Pi.syl	Li.sp	Fr.ex
Ac.pl	Q.ro	C.be	F.syl	C.be	U.sp	P.ab	**V.my**	V.my	L.de	F.syl	Fr.ex	B.sp	C.be	**P.ab**	P.ab	C.be	Ru.sp	Ru.sp	Ru.sp
S.al	U.sp	P.ab	Pi.syl	F.syl	Sa.ni	Ae.sp	Pi.syl	-	Fr.ex	Ac.sp	P.ab	Po.sp	Q.ro	A.al	So.au	T.sp	F.syl	Ca.pr	O.ac
Co.ps	Co.ps	Ru.sp	Ac.sp	Co.av	Ru.sp	**Ac.sp**	So.au	Cx.sp	B.sp	So.au	Sa.ni	Co.ps	Sa.ni	C.be	Q.sp	F.syl	An.ne	Ci.sp	Fra.v
Cr.sp	Cr.sp	Cr.sp	C.be	Ru.sp	Ac.cam	**F.syl**	I.aq	Dry.sp	Ae.sp	An.ne	Ac.sp	Cr.sp	Ac.ps	Fr.ex	Ac.sp	Q.sp	Dry.sp	Fab	Dry.sp
Co.av	All.urs	Ro.co	Fra.v	Sa.ni	C.be	C.be	Ru.sp	Bl.spi	V.my	Con.m	Fr.ex	E.eu	Co.av	Cr.sp	Fra.v	C.be	Bry.sp	Vio.sp	Bry.sp
So.au	Pa.aq	F.syl	He.sp	Cr.sp	F.syl	P.ab	Ac.ps		A.al	O.ac	C.be	Ru.sp	Fr.ex	Ru.sp	Ur.sp	Ac.sp		Cal.p	Jun
Li.sp	An.ne	C.be	Bry.sp	Ac.ps	An.ne	Ae.sp	**O.ac**		So.au	Im.pa	Ac.cam	Sa.ni	Ac.ps	Sa.ni	Ar.ma	Fr.ex		Fra.v	Cy
G.sp	Poa	**An.ne**	Dry.sp	C.be	Ra.fi	So.au	G.sp		Ru.sp	G.od	**All.urs**	An.ne	Ac.cam	Co.av	Cx.sp	Pa.aq		**Poa**	Cx.sp
He.sp	Cx.sp	St.ho		All.urs	O.ac	Ra.fi	Dry.sp		Ac.ps	Cx.sp	An.ne	Poa	All.urs	Li.sp	Poa	An.ne		Jun	
Bry.sp	He.sp	Ra.fi		La.sp	All.pe	G.sp	Bry.sp		O.ac	Dry.sp	G.od	Cx.sp	An.ne	Ru.id	Dry.sp	Ra.fi		**Bry.sp**	
	Bry.sp	Vio.o		G.od	G.sp	Lu.sp			All.pe	Bry.sp	La.ga	He.sp	Cal.p	**O.ac**	Bry.sp	Ar.ma			
		Poa spec		G.ap	La.ga	He.sp			Fra.v		Ur.sp	Dry.sp	Ra.fi	Vio.sp		Con.m			
		Bry.sp		Pa.aq	Vio.sp	Bry.sp			He.sp		Cal.p		Im.pa	Fra.v		Poa			
		He.sp		Ar.ma	Ch.sp				Dry.sp		Bry.sp		La.ga	G.sp		Cx.sp			
		Dry.sp		Ur.sp	Cx.sp								Ur.sp	Fab					
				Jun	Bry.sp								Ar.ma	Poa					
				Poa									All.pe	Cy					
				He.sp									He.sp	Dry.sp					
				Bry.sp									Bry.sp	**Bry.sp**					

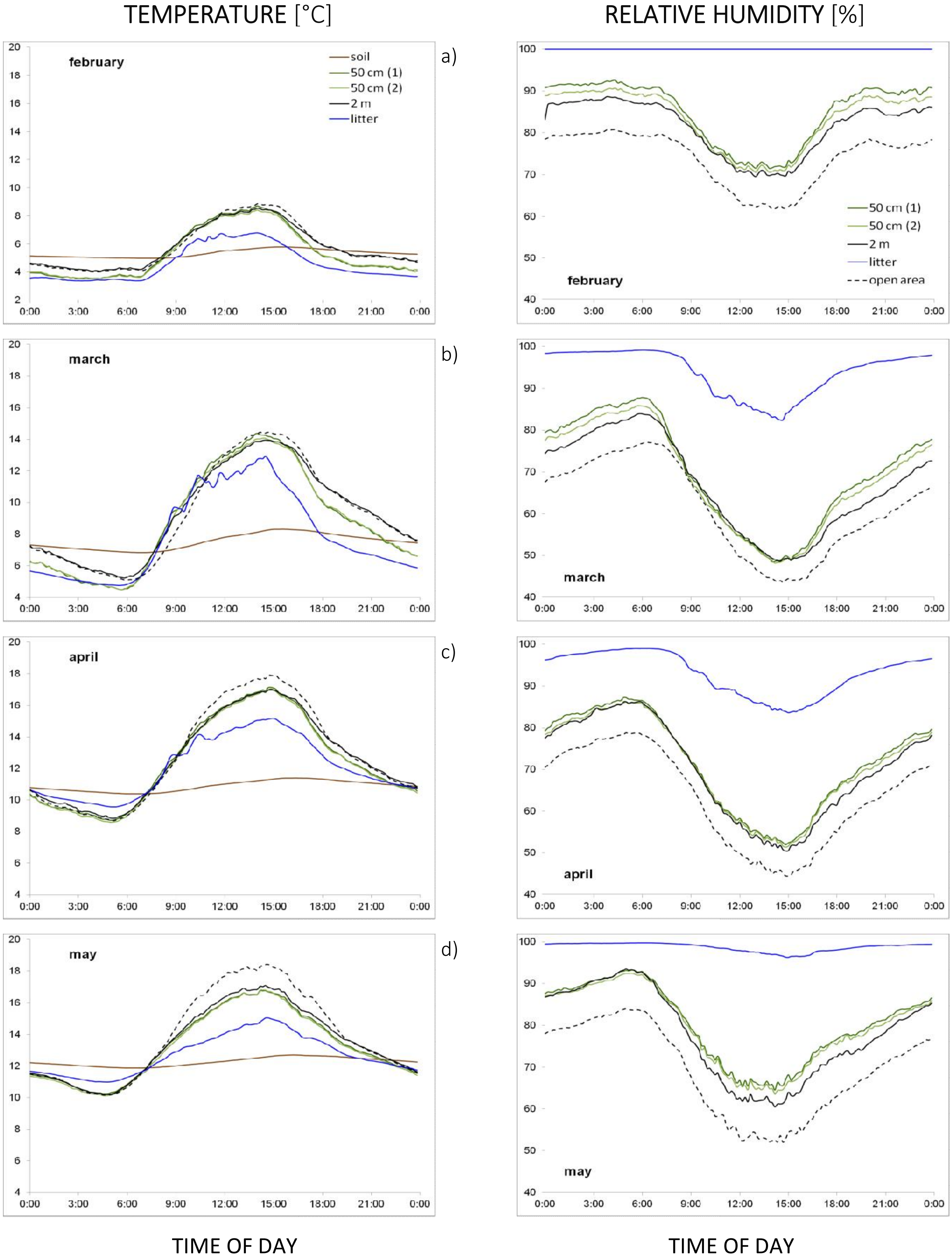
Layer
tree
shrub
herbal
grass/moss/ivy

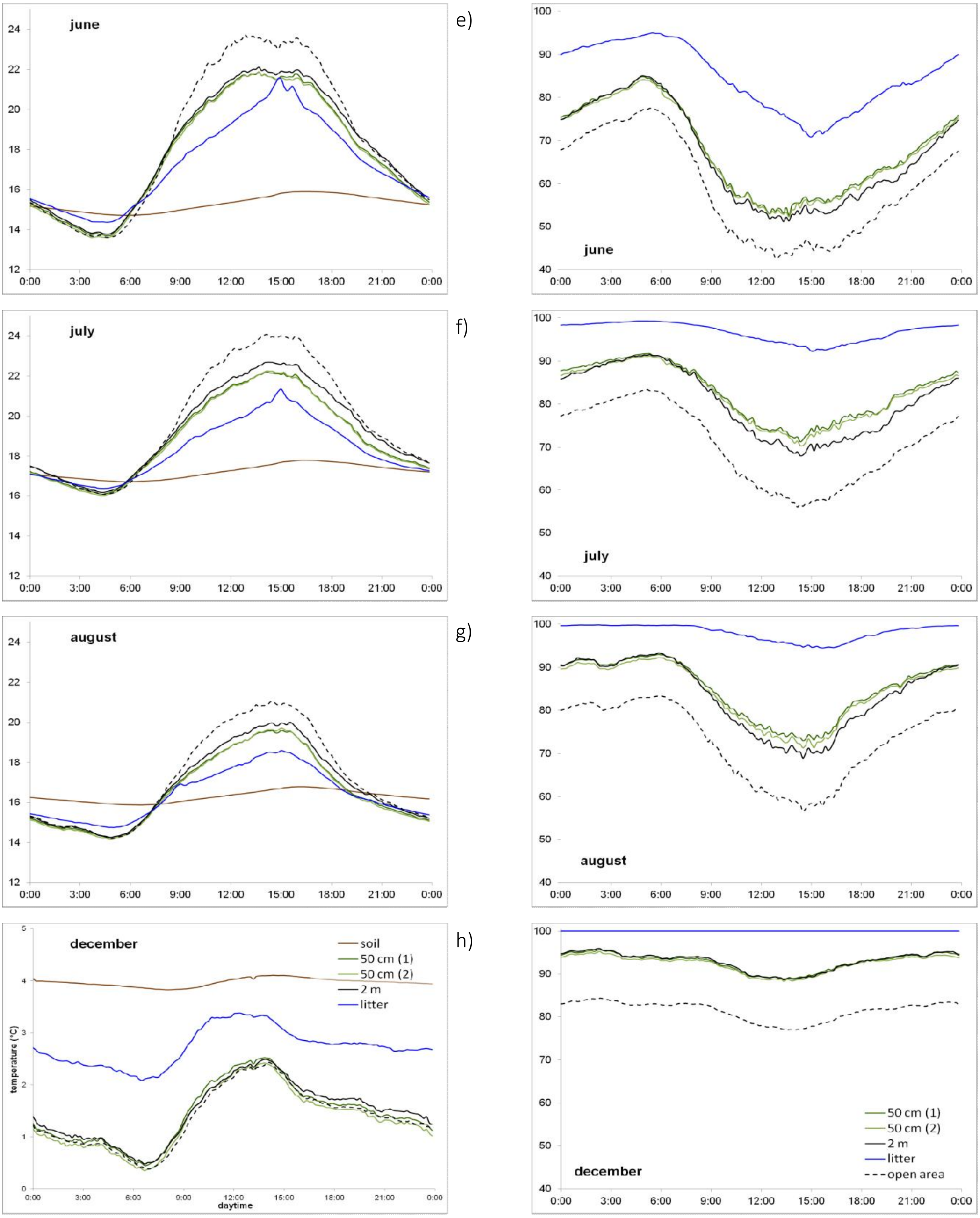
Plant species are separated per vegetation layer for every study site. Study sites are grouped by their ecological landscape; sites located in the border zone of two ecological landscapes (transition area/trans.a.) are set in between. Dominant species are labeled in bold characters. The list is not exhaustive; its focus is on frequently occurring species and indicator plants.

Schwäbische Alb			*Alpenvorland*	
DS	**GH**	**WP**	**FN**	**WU**
F.syl	**P.ab**	**F.syl**	F.syl	B.pu
Ac.sp	F.syl	P.ab	Ac.sp	P.ab
C.be	**A.al**	Ac.sp	C.be	S.sp
Q.sp	Pi.syl	L.de	Pi.syl	Po.sp
Fr.ex	Ac.ps	Fr.ex	P.ab	S.au
Cr.sp	Fr.ex	Pi.syl	B.sp	Co.av
Sa.ni	Ac.ps	F.syl	Al.gl	Li.sp
Be.sp	Ru.sp	Cr.sp	Q.ro	Vi.op
Lo.sp	Ru.id	**An.ne**	Ac.sp	Pr.pa
An.ne	P.ab	Con.m	F.syl	Pa.aq
Ra.fi	F.syl	Vio.sp	Ru.sp	Fi.sp
Im.pa	Co.av	Im.pa	P.ab	Cal.p
Vio.sp	A.al	G.od	I.aq	Ang.sp
La.sp	An.ne	Ac.sp	Fr.ex	Ir.sp
Ar.ma	O.ac	Lu.sp	O.ac	Eq.sp
Pol.sp	Fra.v	He.sp	All.pe	Aj.re
La.ga	G.sp	Bry.sp	Fra.v	Ra.sp
La.sp	Al.sp		La.sp	Phr.au
Poa	Poa		Jun	Ph.ar
	Pt.aq		Poa	**Cx.sp**
	Dry.sp		Cy	**Bry.sp**
	Bry.sp		Dry.sp	
			He.sp	
			Bry.sp	

tree species	***abbr***	**shrub species (2)**	***abbr***	**herb species (2)**	***abbr***
Abies alba	A.al	Crateagus spec	Cr.sp	Galium aparine	G.ap
Acer platanoides	Ac.pl	Euonymus europaeus	E.eu	Galium odoratum	G.od
Acer pseudoplatanus	Ac.ps	Ilex aquifolium	I.aq	Galium spec	G.sp
Acer spec	Ac.sp	Ligustrum spec	Lig.sp	Impatiens parviflora	Im.pa
Aesculus spec	Ae.sp	Lonicera spec	Lo.sp	Iris spec	Ir.sp
Alnus glutinosa	Al.gl	Prunus padus	Pr.pa	Lamium galeobdolon	La.ga
Betula pubescens	B.pu	Rosa corymbifera	Ro.co	Lamium spec	La.sp
Betula spec	B.sp	Rubus idaeus	Ru.id	Meliotus spec	M.sp
Carpinus betulus	C.be	Rubus spec	Ru.sp	Oxalis acetosella	O.ac
Fagus sylvatica	F.syl	Salix aurita	S.au	Paris quadrifolia	Pa.aq
Fraxinus excelsior	Fr.ex	Sambucus nigra	Sa.ni	Polygonatum spec	Pol.sp
Larix decidua	L.de	Sorbus aucuparia	So.au	Ranunculus ficaria	Ra.fi
Liriodendron tulipifera	Li.tu	Vaccinium myrtillus	V.my	Ranunculus spec	Ra.sp
Picea abies	P.ab	Viburnum opulus	Vi.op	Stellaria holostea	St.ho
Pinus sylvestris	Pi.syl	**herb species (1)**		Urtica spec	Ur.sp
Pinus spec	Pi.sp	Ajuga reptans	Aj.re	Viola odorata	Vio.o
Populus spec	Po.sp	Alchemilla spec	Al.sp	Viola spec	Vio.sp
Populus tremula	Po.tr	Alliaria petiolata	All.pe	**grass / moss / ivy**	
Prunus avium	Pr.av	Allium ursinum	All.urs	Blechnum spicant	Bl.spi
Quercus petraea	Q.pe	Anemone nemorosa	An.ne	Bryatae spec	Bry.sp
Quercus robur	Q.ro	Angelica spec	Ang.sp	Carex spec	Cx.sp
Salix alba	Q.sp	Arum maculatum	Ar.ma	Cyperaceae	Cy
Salix spec	S.al	Caltha palustris	Cal.p	Dryopteris spec	Dry.sp
Tilia spec	S.sp	Cardamine pratensis	Ca.pr	Hedera spec	He.sp
Ulmus spec	T.sp	Chrysosplenium spec	Ch.sp	Juncaceae	Jun
Ulmus spec	U.sp	Cirsium spec	Ci.sp	Luzula spec	Lu.sp
shrub species (1)		Convallaria majalis	Con.m	Phalaris arundinacea	Ph.ar
Acer campestre	Ac.cam	Equisetum spec	Eq.sp	Phragmites australis	Phr.au
Berberis spec	Be.sp	Fabaceae	Fab	Poaceae	Poa
Cornus spec	Co.ps	Filipendulan spec	Fi.sp	Polypodiopsida spec	Pol.sp
Corylus avellana	Co.av	Fragaria vesca	Fra.v	Pteridium aquilinum	Pt.aq

C6– MICROCLIMATE - DAILY COURSES AT A MONTHLY DATA BASIS (MB)





Diurnal temperature course at different forest zones on the basis of the monthly mean of 10 minute values, using the example of Michaelsberg (MB). This humus is dominated by beech litter and structured in layers of different decomposition level (= noder/raw humus).

C7– LITTER LAYER – COMPARISON OF TWO HUMUS TYPES

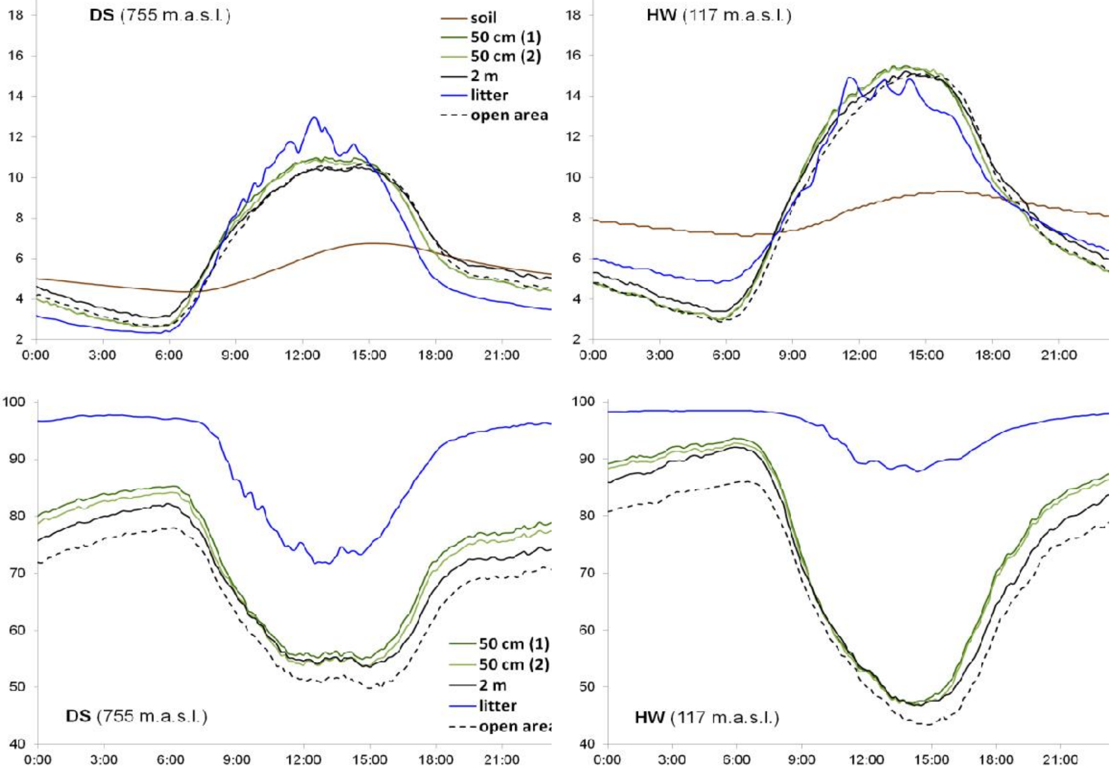
Daily temperature courses, March 2014

MULL HUMUS

(Drackenstein)

MODER / RAW HUMUS

(Hardtwald)



EIGENSTÄNDIGKEITSERKLÄRUNG

Bei der eingereichten Dissertation zum Thema „Habitat ecology and microclimatic influences on the distribution of *Ixodes ricinus* ticks in Baden-Württemberg” handelt es sich um meine eigenständig erbrachte Leistung.

Ich habe nur die angegebenen Quellen und Hilfsmittel benutzt und mich keiner unzulässigen Hilfe Dritter bedient. Insbesondere habe ich wörtlich oder sinngemäß aus anderen Werken übernommene Inhalte als solche gekennzeichnet.

Karlsruhe, den 1. Juni 2016
Denise Heike Böhnke