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„Ecology of the ant community on the alpine tree line ecotone"

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Elia Guariento BSc

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Abstract

Alpine ant communities are poorly studied from an ecological perspective, even in central Europe. At 5 mountains situated in northern Italy, the upper montane forest, the tree line ecotone and the alpine environment directly above the tree line were investigated focusing on ant community composition, resource usage, and possible change of stable isotope signatures over the ecotone. In particular the effect of the tree line ecotone, characterised by an abrupt change in the conditions that limit life, on the ecology of ants was expected to leave a clear signal in ant assemblages. The alpine grassland belt just above the tree line represents the upper distribution limit of most ant species in Europe.

In this study I obtained first insights into the ant communities and how they change over the alpine tree line ecotone. Fourteen different ant species were identified. Elevated species richness was found directly at the ecotone, along with higher functional diversity of the ant community. Both these results were mostly driven by a higher abundance of Myrmicinae species on the ecotone. In the forest a larger potential species pool was detected, but species packing per site was denser at the ecotone. Wood ants dominated in the forest, whereas the subordinate alpine slave-ant (*Formica lemani*) was numerically dominant in the alpine grassland. Wood ant abundance was found to significantly correlate with tree cover (r=0.79) and shrub cover (r=0.80) in the alpine setting. Further, a constrained ordination of the ant community (with exclusion of the wood ants) revealed that shrub cover, soil humus content (inferred from Landolt indicator values) and the density of wood ants influenced significantly the presence and abundance of all other ant species. The feeding preferences were investigated experimentally using a set of baits. This revealed a high preference for sugar (30% of the visited baits) and for sugar-amino acid mixture (32%) over a mixture of multiple amino acids (15%), sodium chloride (16%), lipids (1%) and water (4%). *Formica lemani* was responsible for 77% of bait visitations, wood ants for 29% and all the Myrmicinae subfamily for just 15%. Further, stable isotope analysis corroborated the results of the baiting experiment showing a trophic change among two of the dominant ant species (*F. lemani* and wood ants), with a higher (i.e. more predacious) trophic position at the alpine sites and a lower one in the forest. This pattern was most probably driven by the changing presence of plant sucking homopterans that maintain trophobiotic relationships with ants, which provides a consistent source of plant derived carbohydrates. Both the presence of aphids and the frequency of ant interaction with plant sucking insects decreased moving from the forest to the tree line and even more so into the alpine environment. This trophobiotic interaction most likely influenced the entire community composition and changed resource limitations across the ecotone. The presence of wood ants negatively influenced the number of ground active spiders, but not of ground beetles.

Zusammenfassung

Die Ameisengemeinschaften alpiner Lebensräume sind noch wenig untersucht, auch in Mitteleuropa. Der hochmontane Nadelwald, das Waldgrenz-Ökoton und der alpine Rasen unmittelbar oberhalb der Waldgrenze wurden hier mit einem Fokus auf die Ameisengemeinschaften an 5 Bergen in Norditalien untersucht, und zwar mittels Erfassung der Ameisenarten, durch Anbieten standardisierter Köder und mit Hilfe der Analyse stabiler C- und N-Isotope. Insbesondere wurde der Effekt des Ökoton auf die Ameisen untersucht, da dieser eine abrupte Änderung in den Faktoren darstellt, die das Leben insgesamt limitieren und einen Einblick in die möglichen Beschränkungen für Ameisen liefern könnte, zumal der alpine Rasen die obere Grenze des Vorkommens vieler europäischer Ameisenarten ist.

In dieser Studie wurden erste Einblicke gewonnen, wie die Ameisengemeinschaft zusammengestellt ist und wie sich diese über die Waldgrenze hinweg verändert. Insgesamt wurden 14 verschiedene Ameisen Arten vorgefunden. Ein höherer Artenreichtum wurde an den Standorten direkt am Ökoton vorgefunden, zusammen mit einer höheren Funktionellen Diversität. Beide Ergebnisse waren stark von der höheren Inzidenz von Arten der Myrmicinae beeinflusst, die an der Waldgrenze deutlich häufiger vertreten waren. Im Wald wurde ein größerer Artenpool vorgefunden, das gemeinsame Vorkommen von Arten an einem Standort war jedoch höher an der Waldgrenze. Die Ameisengemeinschaft veränderte sich über den Gradienten, sodass im Wald die dominante Rote Waldameise vorherrschte und im alpinen Rasen die subdominante Alpine Sklavenameise numerisch dominant vorkam. Die Dichte der Rote Waldameise korrelierte stark mit der Anzahl an Bäume (r=79) und im alpinen Bereich mit der Deckung an Sträucher (r=80). Eine dickte Gradienten Analyse der Ameisengemeinschaft (ohne der Roten Waldameise) ergab dass die Deckung an Sträucher, Humus-Gehalt des Bodens (von Landolts Zeigerwerte extrahieret) und die relative Dichte der Roten Walameise einen signifikanten Effekt auf die Präsenz und Abundanz der Ameisen Arten haben.

Die Ressourcennutzung und Limitierung wurde experimentell mit Ködern untersucht und ergab, dass zuckerhaltige Köder wie reiner Zucker (30%) und eine Aminosäuren-Zucker Mischung (32%) stärker besucht wurden als nur Aminosäuren (15%), Salze (16%), Lipide (1%) und Wasser (4%). *Formica lemani* war an 77% der besuchten Köder, die Roten Waldameisen an 29% und alle Vertreter der Myrmicinae nur an 15% der besuchten Köder. Des Weiteren wurde mit einer Analyse der stabilen Isotope die trophische Stellung und die Ressourcenlimitierung der Ameisen bestimmt. Eine niedere Stellung in der trophischen Skala der zwei dominanten Arten wurde im Walde vorgefunden. Dieser Effekt ist wahrscheinlich das Ergebnis einer intensiveren Interaktion mit pflanzensaftsaugenden Homopteren (Pflanzenläusen), die im Wald in großer Anzahl vorkommen und einen wichtigen Lieferanten von Pflanzen-Kohlenhydraten für die Ameisen darstellen. Trophobionten und Ameiseninteraktion mit diesen wurden im Wald in großer Anzahl vorgefunden und reduzierten sich allmählich zur Waldgrenze und im alpinen Rasen, wo eine stärker karnivore Ernährung die Ameisenkolonien aufrechterhält. Die Präsenz von Trophobionten beeinflusste – auf dem Weg über die Dichte der Roten Waldameisen – die gesamte Gemeinschaftszusammensetzung der Ameisen und deren Ressourcenlimitierung über den untersuchten Gradienten. Das Vorkommen der Roten Waldameise beeinflusste auch die Anzahl an bodenaktiven Spinnen, jedoch nicht die von Laufkäfern.

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1. Introduction

Ants are nearly ubiquitous in every terrestrial environment and are among the few insects that can be easily recognised to the family level by everyone (Lach et al., 2010). The most striking characteristic of all ants is that they are eusocial insects (Hölldobler & Wilson, 1990): they live in colonies, characterized by reproductive division of labour, that can reach huge dimensions and they often act as ecosystems engineers and keystone species (Stockan & Robinson, 2016). In some ecosystems, ants make up the most important herbivores or they may compete with earthworms in their contribution to soil perturbation. Ants interact with multiple organisms at several trophic levels and thereby function simultaneously as pollinators, seed dispersers, seed predators or top predators (Hölldobler & Wilson, 1990). As a result, ants often provide essential ecosystem functions and services, like pest control, nutrient cycling and decomposition of organic matter (Del Toro et al., 2012; Wills & Landis, 2017).

Ants are ectothermic animals with a thermophile geographic distribution; their centres of origin as well as of current diversity are located in the tropics (Dunn et al., 2009; Fisher, 2010). Distributional limits of some ants, however, reach far into colder biomes like boreal and subarctic coniferous forests, the tundra (Francoeur, 1983; Heinze & Hölldobler, 1994) and also the alpine grassland belts (Dethier & Cherix, 1982). However, only few ant species (in Austria for example less than 20 species; Seifert, 2007) actually occur regularly above the tree line, leaving alpine or arctic habitats among the few terrestrial ecosystems where ants do not play leading roles.

To study the drivers behind distributional borders elevation gradients are often chosen because here massive variation in important life shaping processes can be observed at small distances (Geraghty et al., 2007; Dunn et al., 2009). Those factors are essentially linked to constraints on the duration of the growing season and on net primary production, both linked to the predictable and stark decrease of ambient temperatures at higher altitudes (Körner, 2007). The altitudinal limit of tree growth represents the most obvious effect of increasing environmental stress on ecosystems (Mayor et al., 2017).

The present study concentrates on the tree line ecotone, with focus on the ant community, its interaction with the surrounding environment and how it changes from the upper montane forest to the subalpine shrub and grassland.

Investigations of the ant fauna along ecotones are quite sparse. Some studies done in the tropics on forest-grassland edges found an increased species richness in the ecotone (Pinheiro et al., 2010), while others did not (Kotze & Samways, 2001). In temperate regions studies are even fewer, but Steiner & Schlick-Steiner (2004) also found an increased species richness on a grassland-shrub edge and Dauber & Wolters (2004) observed an increased presence of dominant ants on agricultural field margins all over Central Europe.

Regarding ant assemblages around the tree line in temperate regions very little information is available, but some conclusions can be drawn by combining studies from alpine settings (Reymond et al., 2013) with those from montane forest (Stuble et al., 2013; Spotti et al., 2015). Another possible way to get an idea about the ecotone effects on ants is to look into studies done on the latitudinal tree line (for example in Canada: Francoeur, 1983; Heatwole, 1989). Another possibility is to focus on studies dealing with altitudinal changes of the ant community to understand how constrains change and how ants adapt to them. Several studies addressing changes of ant communities over elevation gradients in temperate regions concentrate on the documentation of species distributions and species richness (e.g. Sanders, 2002; Glaser, 2006;

Szewczyk & McCain, 2016). Far fewer studies focused on the variation with elevation of different (e.g. morphological) traits on individual (Cushman et al., 1993 on latitude) or colony level (Geraghty et al., 2007). One special field of interest in the myrmecological literature has been the apparent high incidence of social parasites at high altitudes (Buschinger, 2009; Dunn et al., 2009). More generally it has been shown also for ants that at higher altitudes biotic interactions (among ants as well as of ants with other biota) become less important in shaping communities, whereas limitation by abiotic factors takes precedence (Stadler et al., 2003; Dunn, Guenard, et al., 2009; Machac et al., 2011; Bishop, 2017). Two recent papers on alpine ant communities in the Alps (Reymond et al., 2013; Spotti et al., 2015) give first ecological insight into the community composition, functional diversity, feeding behaviour, and concomitant changes with elevation. Except of these two studies and several faunistic reports there is a lack of studies dealing with the ecology of alpine ant communities. The present study aims to partially fill this gap.

The alpine grassland represents the altitudinal distribution limit of almost all ant species in Europe (Dethier & Cherix, 1982; Seifert, 2007). Only few (about 20) ant species occur regularly at this elevation and these are often either habitat specialists or very tolerant ones (Machac et al., 2011). The low temperatures characteristic for alpine habitats, the resulting short growing season and the limitation of primary production are probably responsible for the distribution limit of most ant species to be located near the tree line (Sanders et al., 2007; Machac et al., 2011; Bishop, 2017). The differentiation of the factors (temperature vs. primary production) limiting ant presence at high altitude is not completely understood, because they often change concomitantly and are so hard to differentiate. Some studies found that the temperature is the main limiting factor (Sanders et al., 2007; Bishop et al., 2016) and personal experience of baiting (Guariento et al., 2018) indicated that food should not be the limiting factor near the altitudinal border of ants presence, suggesting that primary production is not the main limiting factor. A very exhaustive recent study instead suggests that not just temperature, but a complex interplay of several drivers seems to limit ant diversity on elevations (Szewczyk & McCain, 2016).

According to several authors (e.g. Theurillat & Guisan, 2001; Pauli et al., 2014) alpine environments will be most severely affected by climate and land-use change in the near future. Since temperature is the main limiting factor for the position of the tree line ecotone as well as for the occurrence of ants at high elevations (Dunn et al., 2009), distributions of trees as well as ants on elevation will probably profoundly shift in the next few decades. The tree line ecotone was pointed out as a suited study system to track climate change induced changes on the environment (Kupfer & Cairns, 1996) and ants as suitable organisms to track small-scale effects on edges (Steiner & Schlick-Steiner, 2004). As a consequence, studying the ecology of ant communities on this ecotone might help to set a baseline which is essential to forecast possible effects of climate change on alpine ant assemblages. Several authors (Majer & Kaspari, 2000; Underwood & Fisher, 2006; Fisher, 2010) suggested ants as a suitable target group for monitoring how climate change is affecting animal communities. The advantage of using ants (instead of other, more mobile animals) as indicator organisms is that their colonies are rather long-lived and stationary, similar to plants (Andersen, 1995).

The fact of being stationary implicates that an ant colony must deal with all conditions of the site with very limited spatial movement and possibilities to avoid unsuitable situations (Andersen, 1995). Hence, the existence of a mature ant colony at any given site is a clear indication that all biotic and abiotic conditions were sufficiently met enabling its survival.

Already in the 1990ies, Andersen (1995) developed a functional typology for the ants of Australia and then of North America (Andersen, 1997). No such classification has been done for European ants; but given a rather good knowledge about the biology of most species (see especially Seifert, 2017) a direct computation of functional traits is possible (Arnan et al., 2012). Using functional traits and their mean in a community and showing which traits are over- or under-represented in a given habitat, allows inferences about the effects of the community on the surrounding environment and back (Hooper et al., 2002).

Functional diversity within communities, which can be computed from available trait information, may give interesting insights into the drivers acting on a community. For example, character displacement indicates that assemblage composition is driven primarily by competition whereas trait similarity often reflects environmental filtering (Laughlin et al., 2012). In alpine ants, competition is expected to drive the community composition especially within the forest, where the dominant mound-building species of *Formica* s. str. occur in high density (Vandegehuchte et al., 2017). Abiotic environmental filtering instead is expected to prevail in the alpine grassland where a harsher environment is selecting for fewer, but well adapted species (Machac et al., 2011). In a study combining datasets about ant communities along elevation gradients in temperate regions, Machac et al. (2011) found a decrease in phylogenetic diversity with increasing elevation. This pattern is characteristic for environmental filtering, in contrast to a greater phylogenetic diversity at lower latitudes where interspecific competition seems to be the more predominant force. However, in a study on an afromontane elevation gradient no clear functional pattern (regarding morphological traits) in ants was found (Bishop et al., 2015) and in a follow-up study the authors postulated that physiological traits that allow to cope with different temperatures are the main driver of altitudinal distribution of ants (Bishop et al., 2016). In another study focusing on ants in the European Alps, Reymond et al. (2013) found a similar pattern in functional diversity, as found by Machac et al. (2011) for phylogenetic diversity. A significant decrease with elevation of both phylogenetic and functional diversity suggests a strong habitat filter towards some specific traits that enable survival in harsher environments of morphologically similar and phylogenetically related species.

The analysis of the ant community composition changes is also used to obtain insight in possible drives shaping the coexistence between species, especially when dominant species are present in the system, known to actively shape the occurrence of other ant species (Johansson & Gibb, 2016) and of other taxa (like spiders and ground beetles Kotze & Samways, 2001; Robinson et al., 2016).

Another central aspects of the presence and dominance of ant species in a community is the access to plant derived carbohydrates (Davidson, 2005; Gibb & Cunningham, 2009), normally provided by trophobiotic partners (Stadler & Dixon, 2008). For the ants in the studied system, this interaction was stated as particularly important for the wood ants (Timo et al., 2016 and citations therein). The presence of trophobiotic partners is expected to be highest where trees are present that can sustain a larger proportion of plant lice and decrease with increasing elevation (Stadler et al., 2003).

The changing availability of nutrients (e.g. carbohydrate from trophobiotic partners as described above) over a habitat gradient can influence the feeding habit of animals. The feeding habits of animals can itself exert particularly strong effects on their environment, reflecting habitat characteristics and resource limitations, influencing the presence and abundance of prey populations, and they may cascade both up and down the food web (Brown et al., 2004).

Determining resource use within ant communities is a central aspect of many ant studies (e.g. Blüthgen & Feldhaar, 2010; Kaspari et al., 2012; Orivel et al., 2018). In Europe, most ant species are trophic generalists (Fiedler et al., 2007; Seifert, 2017). The alpine ant species are even all considered to be generalistic, and this plasticity could assist alpine ants to persist in this harsh environment (Bishop et al., 2015; Iakovlev et al., 2017). The resource range of generalist ant species is composed of different fractions of nutrients (Blüthgen & Feldhaar, 2010). The main sources of nutrients for European ants are prey organisms (delivering mainly amino acids and sodium; Kaspari et al., 2008) and plant resources (mainly carbohydrates, retrieved directly from flowers and extra floral nectaries or indirectly through trophobiosis; Fiedler et al., 2007; Stadler & Dixon, 2008). Determining the proportion of used nutrients by omnivores is essential to be able to determine their position within the trophic network (Mooney & Tillberg, 2005). This determination is often performed using stable isotope analysis on carbon and nitrogen isotope fractions that enables to determine the trophic position of a species and the main resource usage (Fry, 2006). Another routinely used way to determine the resource use of ants is to analyse the acceptances of different resources offered as baits (Bestelmeyer et al., 2000). Several studies dealing with resource use along elevation gradient have been conducted in the tropics (e.g. Peters et al., 2014; Orivel et al., 2018) and few also on temperate-zone mountains (e.g. Fowler et al., 2014; Spotti et al., 2015, Guariento et al., 2018). Even if all ant species in the alpine zone essentially forage for the same resources, differences in the response to various baits can be expected. For example, the complementary theory argues that the visitation of a given resource will increase the more this resource is limiting in the respective habitat (Kay, 2002). Therefore, using information on bait visitation it is possible infer by which nutrients a specific species or community is limited (Kaspari & Yanoviak, 2001) or how limitations might change over environmental gradients (e.g. Peters et al., 2014; Orivel et al., 2018). The reduced occurrence of trophobiotic partners on higher elevation (Stadler et al., 2003) is concordant with a higher usage of carbohydrates by ants found in the alpine setting (Spotti et al., 2015; Guariento et al., 2018). In contrast, the use of carbohydrate baits exposed in forest can be expected to be lower, mainly because of the presence of many *Cinara* aphids on conifers, which offer a consistent and reliable supply of carbohydrate for ants (Timo et al., 2016). Overall, I expected that the trophic position of the ant community is lower in the upper montane forest, where due to ample usage of honeydew the ants rather have a "herbivore" diet. In contrast, I expected a higher trophic position in alpine grassland, where limited availability of trophobiotic aphids leads to more opportunistic and predatory feeding habits in ants.

1.1. Overall Hypothesis

My overarching research question was to establish how ant communities and their ecological characters change from upper montane coniferous forest across the tree line ecotone into alpine grassland in the Alps. I tested the following specific hypotheses:

1. Species richness and species composition change between forest and grassland, with largest diversity in forest (where communities are dominated by mound-building wood ants), lowest diversity in grassland (where mound-building wood ants become rare) and highest species richness on the ecotone.
2. Functional trait diversity and trait composition change concomitantly with ant species composition along this environmental gradient.

3. As predicted by compensation theory, ants show a stronger use of carbohydrates in the alpine grassland, but forage more for amino-acid and salt baits in the forest.
4. Stable isotope analysis reveals a stronger role of trophobiosis in mound-building *Formica* s. str. than in *Formica* (Serviformica) *lemani*, and a shift in both subsets of Formica ants towards lower contribution of directly plant-derived nitrogen above the tree line, where honeydew-producing homopterans become scarce.

2. Material and Methods

2.1. Study sites

The sampling was conducted on five slopes of the southern part of the central siliceous Alps, in South Tyrol (Italy). To facilitate comparisons between sites, only south facing slopes were chosen. One slope (M) was located over quartz phyllite, the other over old siliceous bedrock (online source 1). Two slopes were situated within the nature reserve Texelgruppe and three within the National Park Stilfserjoch (Fig. 1; Table 1). I chose to sample within nature reserves or national parks to minimize effects of land use activities (e.g. pasturing and forestry) as much as possible.

The National Park Stilfserjoch was created in 1935, but only in the 1970es stricter regulations and enlargements enabled a better preservation (online source 2).

The nature reserve Texelgruppe was created in 1976 and is delimited in the north by the border to Austria. The younger origin of this park and more intense activities such as summer pasturing and logging have lowered the actual tree line for many elevation meters (online source 3 and 4)
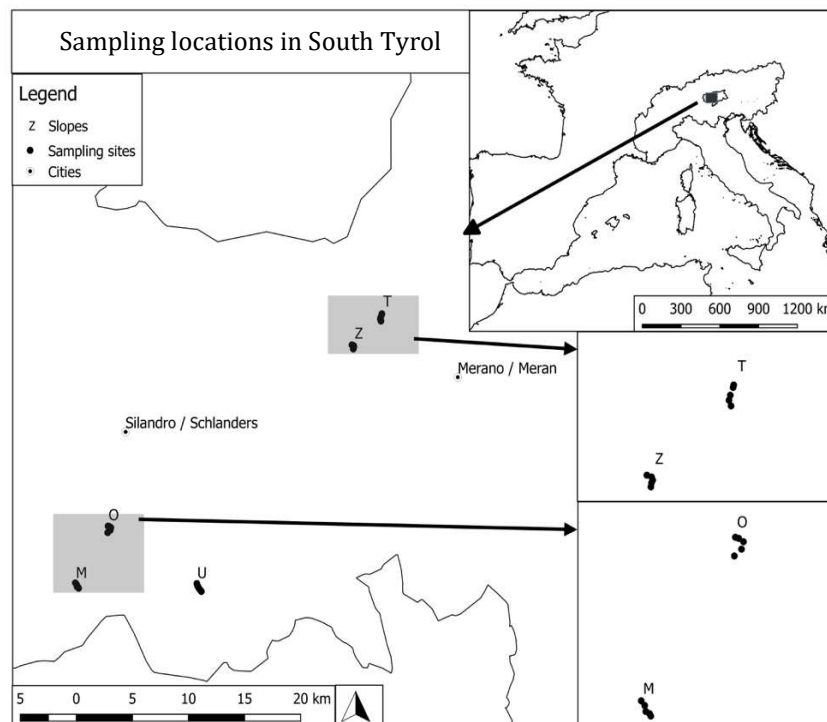
Figure 1. Map of the study sites. The three slopes denominated O, M and U are within the National Park Stilfserjoch, the other two, denominated Z and T, are situated in the nature reserve Texelgruppe.

Each selected slope revealed a gradient of vegetation from subalpine woodland to open alpine grassland interspersed with shrubs, without major changes in exposition and steepness. The tree line itself was in most cases rather clearly defined as a result of management that occurred in the past and still persists in the form of extensive summer pasturing by cattle (Tasser et al., 2001).

On each slope five sites of 800m^2 area were sampled. The sites were selected in reference to the tree line, starting from 100 elevation meters below the tree line and ending 100 elevation meters above. This way, two sampling sites per each slope were located inside the forest, one exactly on the tree line ecotone and two in alpine grassland. The sites were spaced by at least 50 meters in elevation from each other and covered an altitude range from 1935 m (the lowest site) to 2405 m (the highest site). The mean gradient range within each slope was 237 m altitude. The total elevation range was of 470m, with the sites within the National Park (coded as U, O and M) having a higher tree line (about 155 m higher) as the sites within the nature reserve (coded as Z and T; Fig. 2). The lower tree line within the nature reserve is probably a lasting result of more recent direct human intervention (Tasser et al., 2001).

All in all, data were collected on five slopes and 25 different sites, five of which were situated in the ecotone itself, 10 above within the alpine environment, and 10 below within the montane forest. The sampling took place in July and August 2016 and 2017.
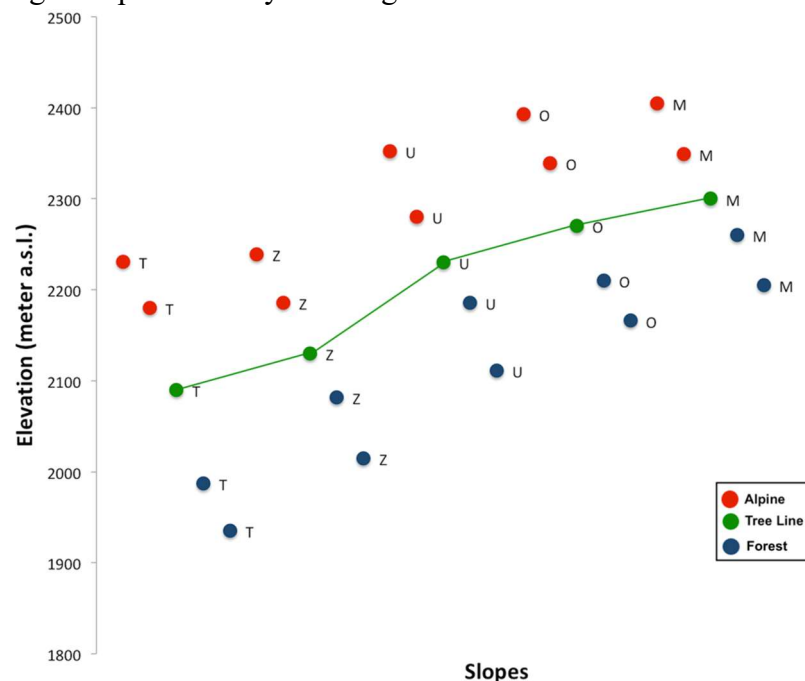
Figure 2. Distribution of the sites in regard to their altitude and to the slope they belong to. The first two (T and Z) where located within the nature reserve Texelgruppe and the last higher three within the National Park Stilfserjoch.

2.2. Field sampling

Applying several different methodologies and approaches can help to get a better picture about ecological effects and allows a more complete coverage of species (Delabie et al., 2000; Hunter, 2001). Following this thought five different sampling methods for ants were implemented simultaneously in this study. In addition, some further taxa (plants, plant lice, ground beetles and ground active spiders) that interact directly or indirectly with ants were surveyed.

The timing of data collection is central to data quality; ideally, sampling should occur when most ant species are active and the highest concentration of foragers can be met outside their nests (Kaspari, 2000). According to Spotti et al. (2015) this occurs in the Alps between 10 and 12 am and ant activity starts to decline significantly after 2 pm. Sampling was therefore set to occur within this rather narrow time frame, but often started at 9 am and ended before 4 pm. The

retrieval of some baits occurred also after 6 pm because of distance and reachability of the sampling locations.

The ecological role of a species in a habitat is related to its abundance (i.e. number of colonies per unit area) and the density of its activity (i.e. number of worker ants foragimg outside the nests). Therefore, both these dimensions were accounted for by using several complementary sampling methods. For the single ant species, as stationary animals, the best measure of their reltaive importance (or best habitat suitanbility) in an environment would be to quantify the density of colonies or nests (Bestelmeyer, 2000). Alternatively to this rather complicated and time-consuming method one might use an activity density measure of the whole community (Bestelmeyer, 2000). In the present study, pitfall traps and quadrat sampling were implemented along with baiting, nest counts and hand sampling (Bestelmeyer, 2000).

2.2.1. Baits

Baits with six different resources following a well-established protocol (e.g. Kaspari et al., 2012; Fowler et al., 2014; Peters et al., 2014; Spotti et al., 2015) were exposed. At each site, five replicates of each bait type (arranged in five transects) were set up, orthogonally to the slope (Fig. 3). Each transect was set at a distance of ca. 10 meters from the neighbouring transects. In the second year two further transects (four on the tree line sites) were placed with the same arrangement on exact the same sampling sites. The baits were offered on the ground in 50ml plastic centrifuge tubes (delivered by Microboss Hightech UG), so that the opening was leaning on the ground. The amount of liquid bait was between 10 and 20 ml soaked in a cotton ball to prevent it from spilling over and to maintain a wet condition throughout the exposition time. Bait resources were: tap water, NaCl (table salt 20 g / 1 L H_2O), sugar (sucrose 200 g / 1 L H_2O), lipids (commercially available pure virgin olive oil), amino acid (L-glutamine 200 g / 1 L H_2O; as offered in Fowler et al., 2014; Peters et al., 2014), mix (100 g sucrose + 100 g glutamine / 1 L H_2O; mixture where found attracting more ants in some studies: Blüthgen & Fiedler, 2004; Kaspari et al., 2012). Baits were exposed for at least 3 hours. Then all ants visiting the baits were counted and voucher specimens were taken for further species determination.

2.2.2. Pitfall traps

Pitfall traps are one of the main methods for surveying ground active ant communities (Bestelmeyer, 2000) and especially in the Alps they may yield large catches (Tista & Fiedler, 2011). Ten pitfall traps (plastic tube with of 3 cm diameter opening and 7 cm deep) were placed on each site, spaced by ca. 10 meters and exposed for three days (on slope U due to adverse weather condition the exposition lasted just 2 days, but without major effect on the numbers of recorded specimens and species; Fig. 3). The traps were partially filled with a baiting liquid composed of 1:1 mixture of rum and honey and few drops of a detergent to reduce surface tension (demonstrated to be effective in Tista & Fiedler, 2011). The traps were similar in size as already used pitfall traps for ants (e.g. Tista & Fiedler, 2011). They were placed so that the soil surface and the trap edge were evenly connected. A metal net with meshes of 1.5 cm width were placed on top and fixed in the soil to hinder other bigger animals or debris to fall into the traps. A tiny plastic roof was also fixed on top to shed them from possible rain.

Since pitfall traps inevitably collect other epigaeic arthropods as by-catch, sampled specimens of other groups that might compete with ants (like ground active spiders and ground beetles) were also counted and determined.

2.2.3. Quadrat sampling

A further method to quantify species composition and activity of forager ants on the ground is quadrat sampling (Bestelmeyer, 2000). Here, a fixed frame of 50 cm x 50 cm in size is set on the ground and all ants within or entering the frame in a fixed time period (10 minutes) were counted and identified. At each sampling site this method was performed three times at randomly chosen subplots covering the major structural vegetation elements present at each site (Fig. 3). It was performed simultaneously with the baiting method in summer 2016. This method can give insight into the relative importance of ants as foragers on the ground and allows also to sample species that might not occur on baits (Bestelmeyer, 2000). Since no clear question was formulated regarding the activity and the method were performed just once the data were not further evaluated except for the incidence of ants found applying this method.

2.2.4. Colony sampling

Colony sampling method was performed inside a fixed range (10m x 10m, 100 m^2) where all colonies of smaller ants are counted, yielding an approximate value of colony density per area (Bestelmeyer, 2000). For the bigger species (like mound-building *Formica* s. str.) all nest mounds were counted inside the whole sampling area (800 m^2). This method was performed only once for each site and was thought to give insight into the distribution of colonies. The problem of polydomous colonies was taken into account by considering each nest as one unit if the distance to the next nest of the same species was within 2 meters distance (only for bigger species). Since colony sampling on such a small area may not provide reliable estimation of nest density for bigger ants (Seifert, 2017), this data was not further evaluated in statistical models, except for the incidence of ant species found only through applying this method.
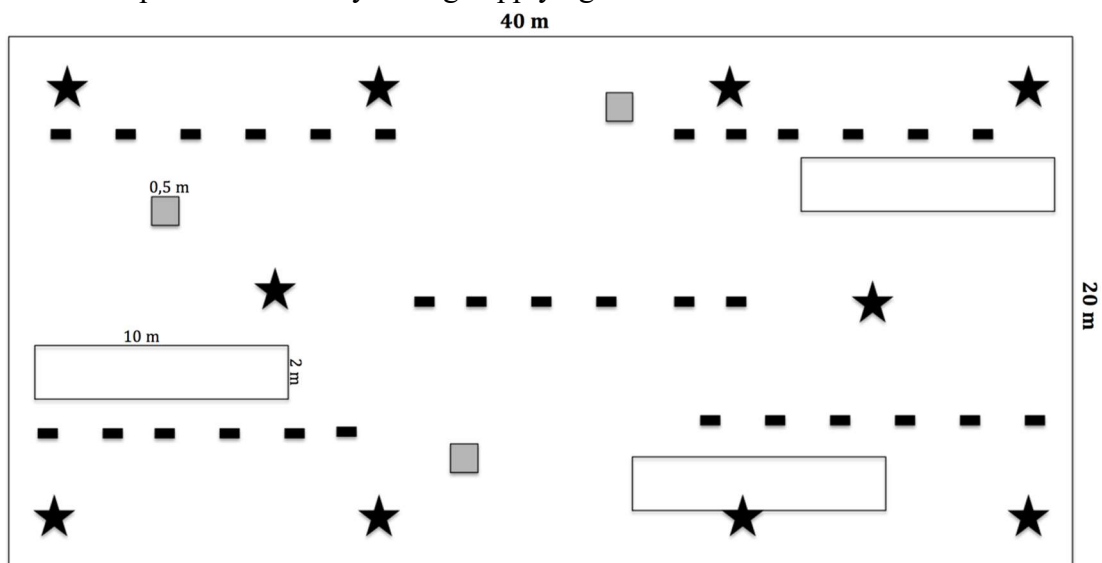

Figure 3. Schematic design of the ant sampling performed within each site. The 10 stars denote the approximate location of pitfall traps; the black dashes the individual baits arranged in transects; the grey quadrats where activity sampling took place; the white 10m x 2m areas are the transects where homopterans were sampled.

2.2.5. Hand sampling

Additionally, hand sampling was performed (Bestelmeyer, 2000) to look for species that might have a cryptic lifestyle and could be missed with the other methods. A searching time of 20 minutes at each sampling site enabled a standardisation of this method.

2.2.6. Sampling of plants

As many plant species as possible occurring within each plot were recorded in the summer 2017 (Tab. D in Appendix). Most of the species were determined at the site using Fischer et al. (2008) and Jäger & Werner (2011). If necessary, herbarium vouchers were taken to be determined afterwards by specialist botanists (see acknowledgements). Subsequently, some indicator values following Landolt (1977) were extracted for each plant species and the median for each site was computed to characterise the abiotic conditions of each site. The indicator values derived from the Landolt list were Humus, Temperature, Light, Nitrogen and Reaction value.

2.2.7. Sampling of Sternorrhyncha

The sampling of trophobiotic partners of ants took place in the summer 2017, always within the same sampling plots as for all other methods. Single animals and entire aggregates were searched on three subplots 10 m x 2 m within each site (Fig. 3). Leaves and branches of the vegetation up to 2 m above ground were carefully searched for ca. 15 min per subplot. Within forest only vegetation that could be reached from the ground was checked. Within each subplot, each tree was recorded and if there was ant visitation on it, indicating ant-attended homopterans in the canopy that could not be reached.

2.3. Environmental variables

The daily mean temperature was measured at 10 cm soil depth (with a hand thermometer with 10 cm long stick with the sensor placed on top) to possibly avoid major daily fluctuations. Other weather parameters were noted, such as air temperature, measured in the shade at 1 m above ground with the same thermometer. Site characteristics like the coverage of stone and bare soil (in %), the vegetation height, the total coverage (in %) of the major plant life-forms were noted for each site. A habitat classification following Wallnöfer et al. (2007) was also done.

2.4. Species identification

Ant voucher specimens collected were determined morphologically to species level using the keys of Seifert (2007) and Wagner et al. (2017), with the help of a stereo-microscope (90-fold magnification) connected to a computer. DNA-barcoding to determine genetically the species identity and validate the morphological determination of some samples was performed at the University of Vienna. The ground beetles were determined by Dominik Rabl MSc using the key of Freude et al. (2004).

2.5. Stable isotope analysis

Stable isotope analysis (C^{13} and N^{15}) was performed to obtain insights into the trophic position occupied by the ant species within the community (Fiedler et al., 2007) and if this position might change across the tree line ecotone. Only samples of *Formica lemani* and *Formica* s. str. were analysed. The analysis was performed in the Department of Microbiology and Ecosystem Research, University of Vienna.

According to Fiedler et al. (2007) the effect of altitude on changes of N^{15} signatures must be taken into account because it could influence drastically the outcome. Similarly, variation in habitat or bedrock could also shape isotopic composition. We accounted for these possible effects by collecting on each site samples of several dominant plant species (a mixture of three to five species of both woody and herbaceous plants). We suspected that homopterans and other herbivores predominantly feed on these plants. We then compared the difference in nitrogen isotope concentration between plants and ants with the reference signature of typical herbivores like Orthoptera and Lepidoptera larvae, which were also randomly collected on the plots.

All sampled materials destined for isotope analysis were directly killed and stored in 96% alcohol in case of the animals, and immediately dried in silica gel in case of plants. Mixed samples consisting of 5 to 8 individual worker ants belonging to 3 to 5 nests per location were taken and the abdomen dissected to prevent possible food stored in the gaster to influence the isotope concentrations (Blüthgen et al., 2003). For the herbivores whole Lepidoptera larvae or the legs of grasshoppers where taken in mixed samples (from 2 to 6 individuals). All samples where dried in an oven at 50°C for 46h and homogenized using a grinding mill. Then the samples were weighted (0.5–0.8 mg for animals; 1.5–2 mg for plant materials) and packed in tinfoil before being analysed. All further analysis were made in the same manner as described in the method section of Adams et al. (2016).

2.6. Statistical methods

2.6.1. Species richness, and accumulation curves

Observed species richness was analysed by mixed model ANOVA in the R environment (R Development Core Team, 2017). Slope identity was modelled as random factor to account for spatial autocorrelation and habitat (with the 3 categories alpine/tree line/forest) as fixed factor. Results were visualised using ggplot2 (Wickham, 2009). The number of ant individuals found in the pitfall traps was analysed in the same manner. Sampling coverage and species accumulation curves were computed using the *R* package *iNext* (Hsieh et al., 2016) with the species incidences with each sampling method (5 methods) on each site (25 sites).

2.6.2. Community composition

Community composition and its drivers were analysed using both constrained and unconstrained ordinations. Bray Curtis distances were used as basis for both computations. For both these analyses a relative abundance of each ant species was computed by summing up the incidences of each ant species with each method on each sampling site. Five different methods of ant sampling were applied and if a species was found by all five methods it received the relative abundance score of five, the maximum possible abundance. This measure considers the ecological importance of species in driving the impact of ants on a specific site. This relative abundance value is depicting both numerous and behaviourally dominant species and might over represent bigger and more active species, but ecologically speaking, this was considered an appropriate scaling of the local prevalence of ant species.

For unconstrained ordinations non-metric multidimensional scaling (NMDS) was performed using the function *metaMDS* in the package *vegan* (Oksanen et al., 2017). Explanatory variables were z transformed (to allow a comparison on the same scale) and post hoc plotted on top of the resulting ordination.

For constrained ordinations the function *capscale* (also in the package *vegan*) was used. Nine explanatory variables were used to build the full model. These variables included some environmental data (such as altitude, habitat type and median Landolt indicator value of the vegetation, derived from the plant species list comprising the values for Light and Humus, shrub cover and the relative incidence of *Formica* s. str.). This relative incidence of the mound-building species within each sampling site was obtained by summing up the incidence on all baits and in all pitfall-traps, normalized by the number of these sampling units (40 per site) to obtain a relative incidence of *Formica* s. str. between 0 and 1.

After constructing a full model to explain species composition by the suite of environmental descriptors, we simplified this model through model selection using a combination of criteria. The Akaike Information criterion corrected for small sample sizes (*AICc*) was used to determine a set of the best models (only *AICc* <2 was considered as improving model fit). Further Akaike weights (Wagenmakers & Farrell, 2004) and R^2 (divided in R^2 and R^2 adjusted with function *RsquareAdj* in *vegan;* Legendre et al., 2011) were used to finally decide which set of variables optimally explained the data.

2.6.3. Functional diversity and Community weighted means

Seifert (2017) collected lots of information about the ecology and distribution of European ant species across a wide geographical range along with the indicator values of vascular plants present on site. For the computation of the community weighted means the relative abundance of the species was used. The trait matrix was compiled using mainly the recently published article mentioned above (Seifert, 2017), supplemented by scores of the competition hierarchy from Savolainen et al. (1988). Altogether, I collected information on 14 different traits describing ecological and eco-morphological characteristic like morphology, nest characteristics, colony founding strategy, feeding habits, foraging behaviour, geographic distribution and dominance aspects of the of all observed ant species (Tab. C in Appendix). The delivered dimensions of functional diversity rely on the publication of Villéger et al. (2008) and Laliberté & Legendre (2010). Both functional diversity values and community weighted means were then analysed between habitats and ecotone with mixed model ANOVAs (function *aov* in R) and depicted with box-plots with ggplot2 (Wickham, 2009).

2.6.4. Baiting experiment

For the baiting experiment only the presence or absence (i.e. 0/1 incidences) of ants on each bait was used for statistical analysis. Generalised linear mixed models (GLMM) were computed with the R package *lme4* (Bates et al., 2015) and function *glmer* with a binomial error structure. For *Formica lemani*, for the subgenus *Formica* s. str. and for the family Myrmicinae separate GLMMs were performed to analyse the feeding decisions of ants on lower taxonomic levels. These groups reflect differences in visitation intensity (abundant and seldom visitors, *Formica* vs. Myrmicinae) and behaviour (dominant and subordinated, *F. lemani* vs. *Fomica* s. str.). AICc (AICc < 2), R^2 (partitioned into marginal and conditional R^2: Nakagawa & Schielzeth, 2013) as well as Akaike weights were computed (Barton, 2018; Wagenmakers & Farrell, 2004) to determine the best variable combination. Zero inflation was excluded for all models. A null model was always implemented and rejected because of worse fit to the data than the optimal model.

2.6.5. Community relationships to other taxa

Using the by-catch of ground beetles and ground active hunting spiders in the pitfall traps, I compared relationships between the abundance of these competitors of ants and the relative prevalence of mound-building *Formica* s. str. in linear regressions. By means of a constrained ordination (function *capscale* in package *vegan*) it was further evaluated if the relative abundance of wood ants influenced the occurrence of ground beetle species.

2.6.6. Trophobiotic associations with Stenorrhyncha

The data about the occurrence of homopteran aggregations and their trophobiotic associations with ants turned out to be very uneven, changing much between the different slopes and sites, only a descriptive evaluation of this data was possible. All associations of plant sucking insects, the plant on which they occurred, the ant species visiting them, and the number of visited trees was recorded.

2.6.7. Evaluation of plant species occurrences

The matrix of vascular plant species occurring on each site was used in two different ways: First, an unconstrained analysis (function *metaNMDS* in *vegan* based on Sørensen dissimilarities) was performed. The first two axes extracted and subsequently used as explanatory variables in the constrained ordination for the ant community. Second, the indicator values (Landolt, 1977) were extracted to describe the sites characteristics and also implemented as explanatory variables in the constrained ordination of the ant community. To evaluate the differences in plant species incidence between habitats and parks an analysis of similarities (ANOSIM, *anosim* function in *vegan*) based on site dissimilarities (function *vegdist* in *vegan* with binomial dissimilarity index) where used.

2.6.8. Isotope data

Both the nitrogen and the carbon stable isotope fraction were analysed combined together with a mixed-model-PERMANOVA in the statistical software Primer7 (Plymouth Routines in Multivariate Ecological Research, version 7; PRIMER-E, Plymouth, UK). A Euclidean distance matrix, based on the isotopic fractions of C and N, was computed and processed using a mixed-effects PERMANOVA (9999 permutations). The different slopes were included as random factor, and the fixed factors sample type (plant, herbivore, and the two ant species) and habitat (alpine, tree line, forest) together with their interaction were tested. The visualisation was done as a scatterplot of the samples based on the C and N isotopes and boxplots to visualise the differences with the altitude changes of the individual isotopes using the package ggplot 2 (Wickham, 2009).

All data about the occurrence of species (ants, ground beetles, spiders, plants and grasshoppers) will be imported into the database "FloraFaunaSüdtirol" (partially online under: http://www.florafauna.it). Voucher specimens will be deposited in the Naturmuseum Bozen.

3. Results

3.1. Site characterization

Characterisation of the sampling sites was performed taking several different measures of biotic and abiotic aspects. Summary site characteristics are presented in Table 1. They allow for a rather clear classification of sampling sites.

Table 1. Synopsis of site characteristics. Mean temperatures (soil and air), cloud cover and wind intensity were recorded simultaneously with the implementation of the baiting and quadrat sampling methods. „Basal area" refers to the sum of area calculated from the diameter of threes with a diameter at breast height greater than 10 cm found in a 100 m^2 area. The different covers in % refers to the whole sampling area of 800m^2 on each site. Light, Humidity, Reaction, Nitrogen and Humus values refers to the median of indicator value of all plant species on a site taken from Landolt (1977). Finally, the reference number from Wallnöfer et al. (2007) refers to the main habitat classification and in brackets to less prominent habitat characteristics within the sampling sites.

Site code	Slope	Habitat	Date of sampling in summer 2016 and 2017	Longitude	Latitude	Exposition	Elevation (m a.s.l.)	Steepness (in %)	Mean air temperature	Mean soil temperature	Cloudcover (%)	Wind (Beaoufort scala)	Basal area	N trees (d>10cm)	Tree cover (%)	Shrubs cover (%)	Rock/stone (%)	Bare soil (%)	Dead wood (%)	Low veg cover (%)	Light value	Humidity value	Reaction value	Nitrogen value	Humus value	Wallnöfer et al. Liste Nr.
Z 1	Zielspitze	alpine	15.07.2016 / 13.08.17	N46°41.359'	E011°01.829'	SSO	2239	62.12	15.75	11.62	30	2	0	0	0	10	10	2	0	80	4	3	1	2	3	41200
Z 2	Zielspitze	alpine	15.07.2016 / 13.08.17	N46°41.331'	E011°01.942'	SO	2186	63.62	14.65	12.65	15	2	0	0	0	7.5	12.5	5	0	75	4	3	1	2	3	41200 (5200-3-4)
Z 3	Zielspitze	tree line	15.07.2016 / 13.08.17	N46°41.283'	E011°01.968'	S	2130	58.5	15.5	10.9	20	1	4392	15	27.5	15	10	2.5	1	62.5	4	3	1	2	3	45210 (62122)
Z 4	Zielspitze	woods	16.07.2016 / 15.07.17	N46°41.241'	E001°01.941'	SSW	2082	91	15.75	8.27	0	0	7040	12	62.5	20	3	15.5	3	45	3	3	1	2	3	62112-22
Z 5	Zielspitze	woods	16.07.2016 / 15.07.17	N46°41.179'	E001°01.926'	SSW	2015	75	16.62	9.6	0	0	4660	9	45	22.5	10	10	3	35	3	3	1	2	3	62113
O 1	Orgelspitz	alpine	19.07.2016 / 29.07.17	N46°33.218'	E010°44.864'	SO	2393	62	19.32	14.8	0	3	0	0	0	42.5	11	1.5	0	45	4	3	1	2	3	5200-4
O 2	Orgelspitz	alpine	20.07.2016 / 29.07.17	N46°33.201'	E010°44.944'	SO	2339	66.5	18.15	13.85	0	3	0	0	0	55	7.5	3.5	0	35	4	3	1	2	3	5200-4
O 3	Orgelspitz	tree line	20.07.2016 / 29.07.17	N46°33.149'	E010°45.060'	SSO	2270	49.25	18.75	12.55	0	0	2022	12	30	55	6	4	2.5	30	4	3	1	2	3	5200-4 (62310-30)
O 4	Orgelspitz	woods	20.07.2016 / 29.07.17	N46°33.033'	E010°45.013'	SSO	2210	55.5	16.25	12.35	0	2	10413	12	60	12.5	15	11	6	52.5	3	3	1	2	5	62310-122
O 5	Orgelspitz	woods	21.07.2016 / 29.07.17	N46°32.926'	E010°44.839'	SSO	2166	58.5	14.2	12.9	95	0	7550	22	50	20	17.5	20	5	35	3.5	3	1	2	4	62310-123
U 1	Ulten	alpine	02.08.2016 / 22.08.17	N46°30.630'	E010°51.043'	S	2352	57.25	12.6	11.17	80	1	0	0	0	45	11	4	0.5	42.5	4	3	1	2	3	42100 (5200-3-4)
U 2	Ulten	alpine	02.08.2016 / 22.08.17	N46°30.519'	E010°51.103'	S	2280	46.25	12.85	12.5	95	1	0	0	0	42.5	5.5	4.5	0.5	50	4	3	1	2	3	42100 (52400)
U 3	Ulten	tree line	03.08.2016 / 22.08.17	N46°30.440'	E010°51.168'	SO	2230	30.25	14.62	14.35	30	0	4243	10	25	35	6	7.5	2	40	4	3	1	2	3	56400 (62310)
U 4	Ulten	woods	03.08.2016 / 22.08.17	N46°30.374'	E010°51.235'	SO	2186	44.5	14.02	11.07	10	0	10453	16	65	30	3	8.5	6	55	3	3	1	2	3	62310
U 5	Ulten	woods	04.08.2016 / 22.08.17	N46°30.279'	E010°51.354'	SSO	2111	57.5	14	11.47	30	1	20219	33	62.5	10	5	35	7.5	35	3	3	1	2	5	62310
T 1	Tschigot	alpine	08.08.2016 / 02.07.17	N46°42.757'	E011°03.916'	S	2231	83.5	16.32	10.6	5	2	0	0	0	0	5	0	0	95	4	3	1	2	3	45220
T 2	Tschigot	alpine	08.08.2016 / 02.07.17	N46°42.719'	E011°03.900'	S	2180	68	15.82	9.77	0	2	0	0	0	12.5	25	0	0	62.5	4	3	1	2	3	56200 (45220)
T 3	Tschigot	tree line	11.08.2016 / 03.07.17	N46°42.598'	E011°03.833'	SSW	2090	80.5	12.07	8.5	40	2	2547	12	37.5	40	10	10	1	45	3	3	1	2	3	62122 (56200)
T 4	Tschigot	woods	18.08.2016 / 03.07.17	N46°42.521'	E011°03.802'	SSW	1987	53.87	13.27	9.37	50	0	9666	23	62.5	12.5	15	3.5	1.5	45	3	3	2	2	5	62112-22 (45120)
T 5	Tschigot	woods	18.08.2016 / 03.07.17	N46°42.433'	E011°03.851'	S	1935	72.25	12.4	11.47	50	0	13955	7	70	10	20	5		55	3	3	1	2	3	62112
M 1	Martell	alpine	22.08.2016 / 15.08.17	N46°30.662'	E010°42.589'	SSO	2405	48.75	15.1	11.87	0	3	0	0	0	50	7.5	5	0.5	40	4	2.5	1	2	3	41200 (56400-2)
M 2	Martell	alpine	22.08.2016 / 15.08.17	N46°30.588'	E010°42.673'	SSO	2349	40.75	15.9	11.17	0	2	0	0	0	40	7.5	3.5	0	52.5	4	3	1	2	3	56400
M 3	Martell	tree line	19.08.2016 / 15.08.17	N46°30.495'	E010°42.700'	SSO	2300	31.87	14.9	13.05	60	2	4861	8	40	45	3	6.5	4	42.5	4	3	1	2	3	56400 (62310)
M 4	Martell	woods	19.08.2016 / 15.08.17	N46°30.461'	E010°42.789'	SSO	2260	38.25	14.85	12.67	60	1	11763	12	55	15	12.5	15	10	50	3	3	2	2	5	62310
M 5	Martell	woods	25.08.2016 / 15.08.17	N46°30.423'	E010°42.820'	SSO	2205	64.5	17.5	12.35	0	0	7116	24	60	5	10	17.5	12.5	45	3	3	1	2	3	62310

Altogether, 98 vascular plant species were determined in the field or later from herbarium specimen at the 25 sampling sites (Tab. E in Appendix).

A non-metric multidimensional scaling (unconstrained ordination) based on Sørensen dissimilarities of all sites based on plant species incidences was performed. Habitat differentiated clearly the alpine setting and the tree line sites from the forest sites. Also park displayed a significant different plant community (Fix. 4 Tab. 2).
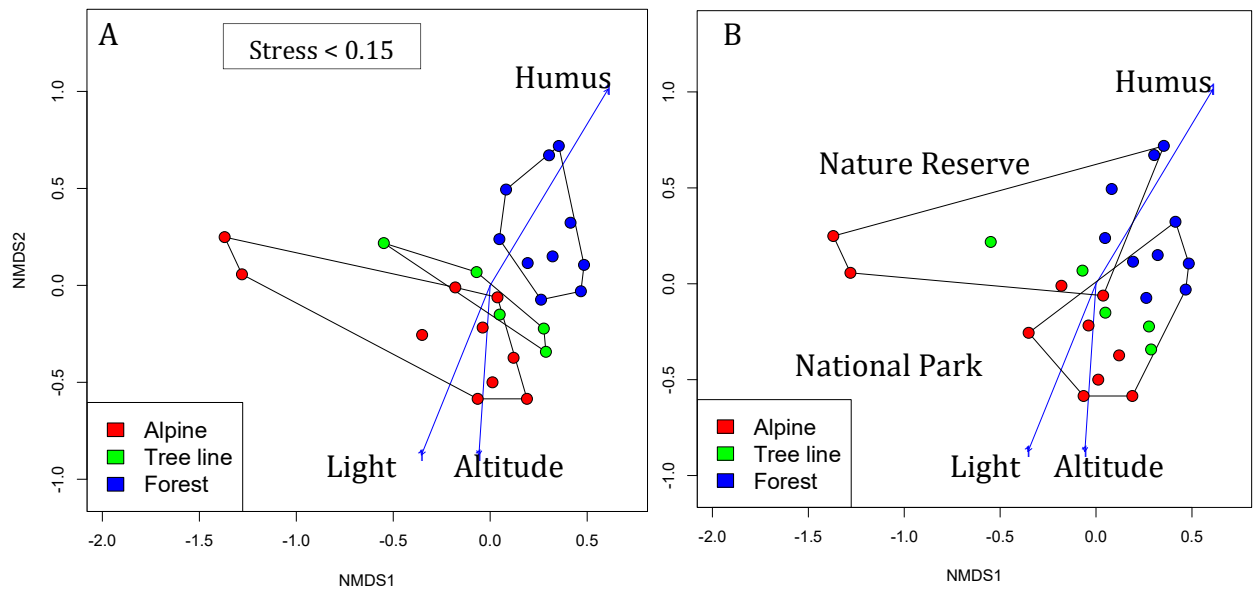
Figure 4. NMDS of the species lists of vascular plants, performed using Sørensen dissimilarities between sampling sites. A clear difference of the three habitat types is recognisable with tree line in between forest and grassland (A). Furthermore, a clear segregation between the sites located on the National Park (lower point cloud) and the ones in the nature reserve (upper) is also recognisable (B). Environmental variables were z transformed before implementation.

Table 2. ANOSIM result of plant community differences between habitat and park.
***: significant at p<0.001.

Grouping factor	p	*R*
Habitat	< 0.001***	0.286
Park	< 0.001***	0.295

Table 3. Pearson correlation matrix (*r* values) among site variables. Significant correlations (p<0.05) in bold face. Most variables correlate with each other. All variables were z transformed prior implementation.

Variables	Altitude (m a.s.l.)	Soil temp	Basal area	Tree cover	Shrub cover	Low veg. cover	Humus value	Light value	Plant NMDS1	Plant NMDS2
Soil temp.	-0.06									
Basal area	**-0.57**	0.31								
Tree cover	**-0.67**	0.12	**0.88**							
Shrub cover	**0.59**	-0.14	**-0.44**	-0.37						
Low veg. cover	-0.04	0.07	-0.26	-0.34	**-0.58**					
Humus value	**-0.45**	-0.10	**0.51**	**0.63**	-0.35	-0.09				
Light value	**0.70**	-0.21	**-0.76**	**-0.87**	**0.40**	0.29	**-0.60**			
Plant NMDS1	-0.15	0.08	**0.58**	**0.61**	-0.19	-0.28	**0.64**	-0.39		
Plant NMDS2	**-0.91**	0.11	**0.55**	**0.67**	**-0.69**	0.18	**0.57**	**-0.71**	0.17	
Formica abundance	**-0.42**	0.07	**0.77**	**0.78**	-0.13	**-0.48**	**0.47**	**-0.65**	**0.54**	0.36

As expected, most of the environmental and biological variables describing the sampling locations correlated with the overall habitat gradient (Table 3; Fig. 12).

3.2. Ant species observed and species richness

All in all, ~23.500 individual ants were retrieved with all sampling methods, which represented 14 different species (Table 5).

Table 5. Ant species found on the sites (letters in the header row denote the slope and numbers the sites on the slope). Habitats marked with different colours: red for alpine, green for tree line and blue for forest.

Species	Z1	Z2	Z3	Z4	Z5	O1	O2	O3	O4	O5	U1	U2	U3	U4	U5	T1	T2	T3	T4	T5	M1	M2	M3	M4	M5	Nr. sites
Camponotus herculeanus L., 1758										X																1
Formica aquilonia Yarrow, 1951									X	X				X					X							4
Formica exsecta Nylander, 1846	X		X								X	X				X	X	X					X			8
Formica lemani Bondroit, 1917	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	24
Formica lugubris Zetterstedt, 1838		X	X	X	X	X	X	X		X	X	X	X	X	X		X	X			X		X	X	X	19
Formica rufa L., 1761														X												1
Leptothorax acervorum Fabricius, 1793	X	X		X	X		X			X		X	X				X	X				X	X	X	X	14
Manica rubida Latreille, 1802		X	X	X	X		X	X		X	X	X	X	X				X		X	X	X	X		X	17
Myrmica lobicornis Nylander, 1846																								X		1
Myrmica lobulicornis Nylander, 1857	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X	X	X	X	22
Myrmica ruginodis Nylander, 1846				X	X																					2
Myrmica sulcinodis Nylander, 1846		X	X		X		X	X	X	X	X	X	X				X	X	X					X	X	15
Tetramorium alpestre Steiner, 2010	X	X	X		X	X	X	X		X		X	X		X			X			X	X	X	X		16
Temnothorax tuberum Fabricius, 1775		X	X	X	X	X	X	X				X	X											X	X	11
Nr. species per site	5	8	8	6	9	5	6	8	4	8	5	8	9	6	6	3	4	7	4	3	8	5	7	7	7	

Species richness was, as expected, higher on the ecotone as compared to the two other habitats. The species number on each site differed from 3 to 8 in the alpine setting, from 3 to 9 in the forest and just between 7 and 9 on the tree line (Table 6; Figure 5).

Table 6. Results of mixed model ANOVA on species richness (species count) across habitats (alpine, tree line, forest). Slope identity was modelled as random factor to account for spatial autocorrelation. **: significant at $p<0.01$.

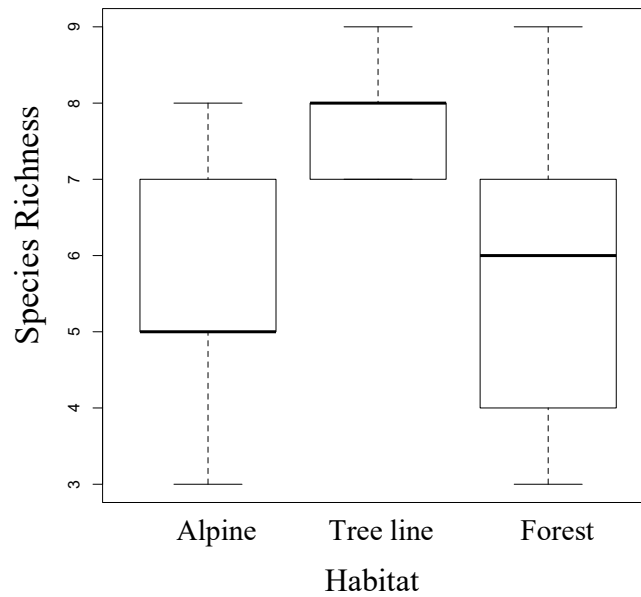|	Degrees of freedom	Sum of squares	Mean sum of squares	F value	p
Species Richness ~ Habitat	2	18.94	9.47	6.30	0.008 **
Residuals	18	27.06	1.50		

Figure 5. Boxplot of species richness are higher on the tree line ecotone than within the other two habitats. Whisker showing the range of observed data, the box depicting the inter-quantile and the line showing the median. The median species number on the tree line was 8 compared to not even 6 in the alpine and forest sites. The ecotone also revealed much less variation in species richness and diversity in comparison to the sites within forest or grassland.

Surprisingly no difference was found in the species number between forest and alpine shrub and grassland. The higher species richness on the tree line was mainly driven by the higher incidence and abundance of Myrmicinae, in comparison to above or below the tree line. *Manica rubida* was found 2.8 times more often on the tree line than in the alpine setting and over 3 times more often than in subalpine forest. Also *Temnothorax tuberum* and *Leptothorax acervorum* occurred more often on the tree line (*T. tuberum*: 2.3 times more than in the alpine environment and 3.2 times more than in forest; *L. acervorum*: 2 times more than in the alpine and 1.5 more than in forest; Fig. 6). Overall *Formica lemani* was the most common ant, followed by *Formica lugubris*. Other quite common species were *Myrmica lobulicornis, Myrmica sulcinodis, Tetramorium alpestris, Temnothorax tuberum* and *Leptothorax acervorum*. Rare species were *Myrmica rubra* found on two sites, and *Formica rufa, Myrmica lobicornis* and *Camponotus herculeanus,* all on just one site (Fig. 6).

The relative pseudo-abundance of ant species was mainly used for multivariate analyses. But it still delivers a clear picture about the dominant ant species and the distribution over the gradient (Fig. 6). The subgenus *Formica* s. str. clearly dominates numerically in the woods and *F. lemani* on the tree line and above. For five Myrmicinae species the abundance is higher on the tree line sites.
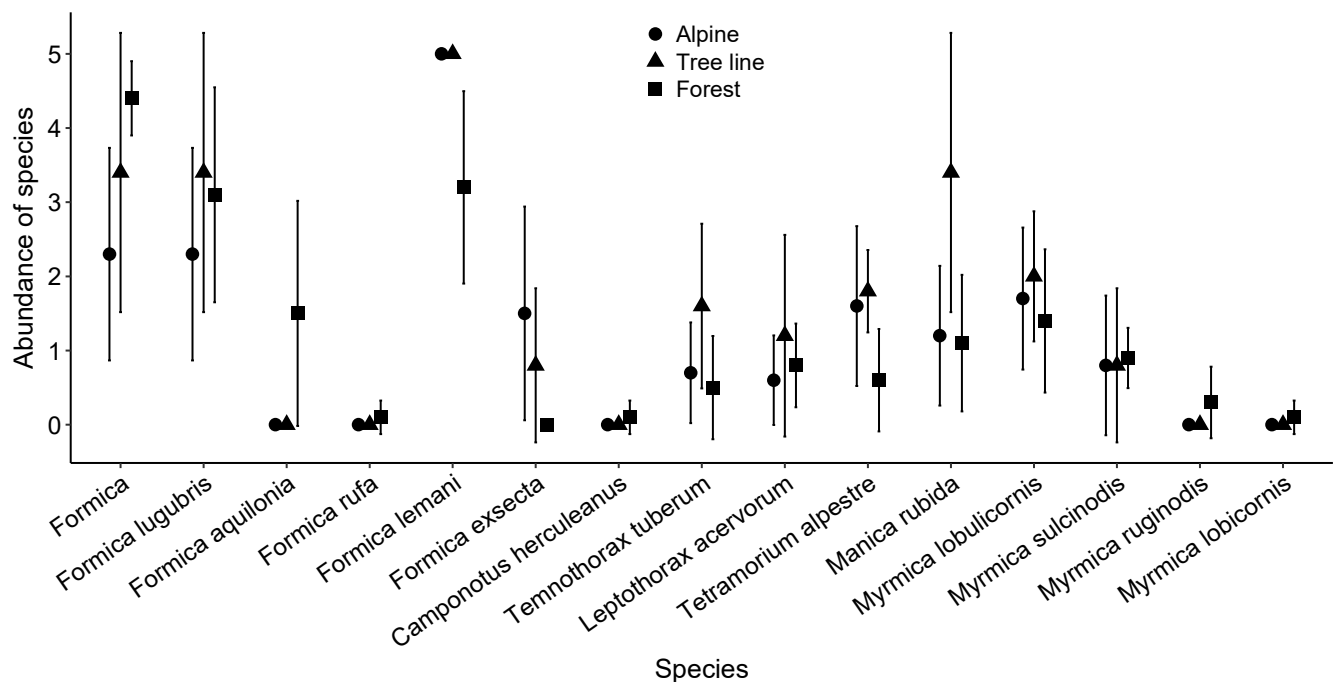
Figure 6. Mean pseudo-abundance of species with 95% confidence intervals split between the three habitats. The maximum relative abundance is five, only reached on average by *Formica lemani* in the alpine setting and on the tree line. The sum of pseudo-abundances of mound-building wood ants (subgenus *Formica* s. str. comprising *F. lugubris, F. aquilonia* and *F. rufa*; first three bars) was added to visualise the overall prevalence of these ants which otherwise tend to exclude each other locally.

Most ant species occurred along the whole gradient at least once (Fig. 7). Five species occurred only within the forest (*Camponotus herculeanus, Myrmica rubra, Formica aquilonia, Formica rufa* and *Myrmica lobicornis*) and just *Formica exsecta* occurred only at and above the tree line. Therefore, the main differences in ant community composition between the habitats and the tree line were due to the relative abundances of each species.
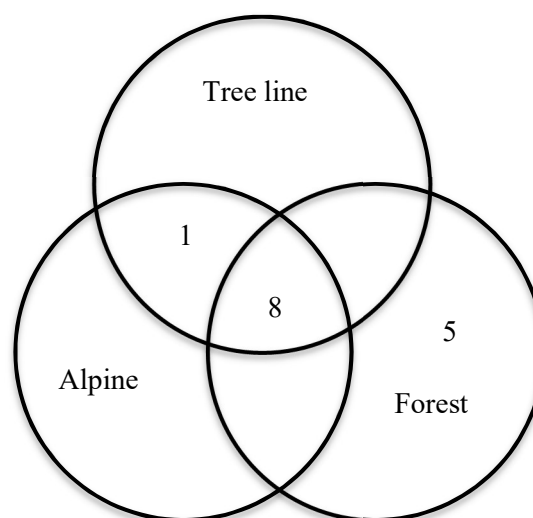

Figure 7. Venn diagram of the occurrence of species in the three habitats. The five species found only in forest were all rare.

The sampling methods yielding most species were pitfall-traps and handpicking, while colony sampling was the least productive (Fig. 8). All in all, the majority of ant species was recorded with all methods and the small differences were due to rare species.
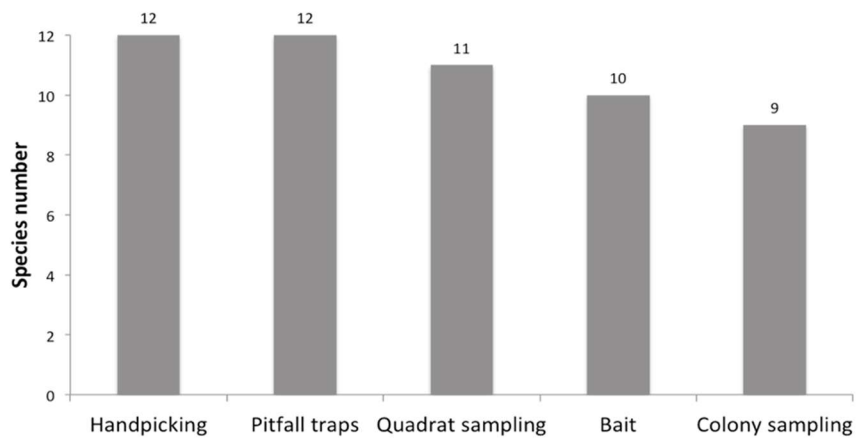

Figure 8. Comparison between sampling methods with regard to the total species number recorded.

Using the *R* package *iNext* (Hsieh et al., 2016) a species accumulation curve was computed to determine how complete the sampling of ants was in the three habitats and how many species where to be expected. For both the tree line and the alpine grassland a very high coverage was obtained, and additional sampling units would likely not contribute further species to be detected. For the forest, in contrast, adding more sampling sites would have delivered a more complete picture and potentially more species (Fig. 9).
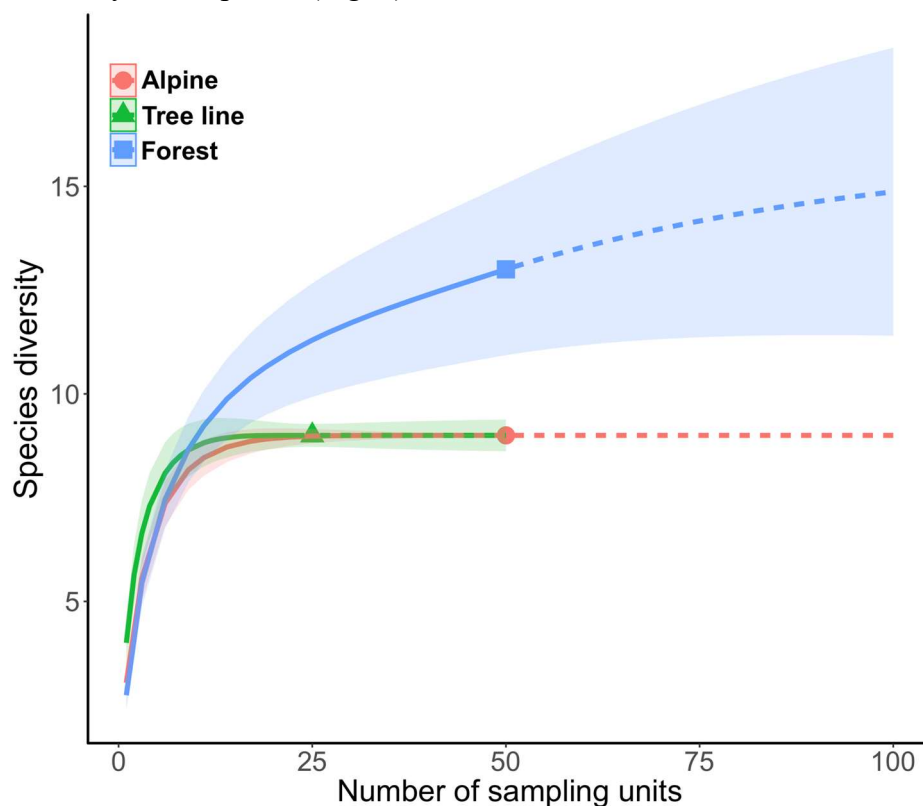

Figure 9. Species accumulation curve as a function of sampling methods applied (5 per site), based on species incidences. In the forest beta diversity was higher and a few ant species have been probably missed.

Ant species richness was not expected to differ between the five slopes. The results showed a fairly even score of all but one slope (T; Tab. 7; Fig. 10).

Table 7. Ant species richness among the five slopes. Slope T and Z were situated in the nature reserve, the other in the National Park. For each computation the habitat was modelled as random factor to focus on possible differences between the slopes. *: significant at $p<0.05$.

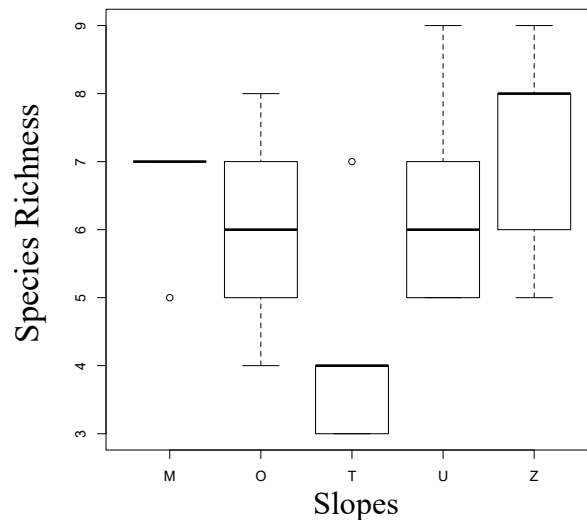| ANOVA	Degrees of freedom	Sum of squares	Mean sum of squares	F	p
Species Richness ~ Slope	4	25.48	6.46	4.30	0.013*
Residuals	18	27.06	1.50		

Figure 10. Comparison of species richness among the five slopes. Slope T in the nature reserve scored significantly lower than the others in species richness. All other slopes (also Z within the same nature reserve as T) showed a similar score.

Table 8. Mixed model ANOVA results for the number of worker ants retrieved from pitfall traps in relation to slope and habitat, respectively. Slope was modelled as random factor in the habitat comparisons, and habitat served as random factor in the ANOVA for the comparison among slopes. Both times the number of workers where square root transformed to met linearity assumption. *: significant at $p<0.05$.

ANOVA	Degrees of freedom	Sum of squares	Mean sum of squares	F	p
Number of Ants ~ Habitat	2	265	132.8	2.07	0.155
Residuals	18	1153	64.1		
Number of Ants ~ Slope	4	1065	266.2	34.15	0.015*
Residuals	18	1154	64.1		

The number of worker ants retrieved from the pitfall traps was 3 times higher on all the slopes situated in the National Park than in the nature reserve (Tab. 8; Fig. 11). Further the number of ants retrieved from the three different habitats showed a higher worker ant activity density in forest and a weak tendency to a lower density on the tree line ecotone (Tab. 8; Fig. 11).
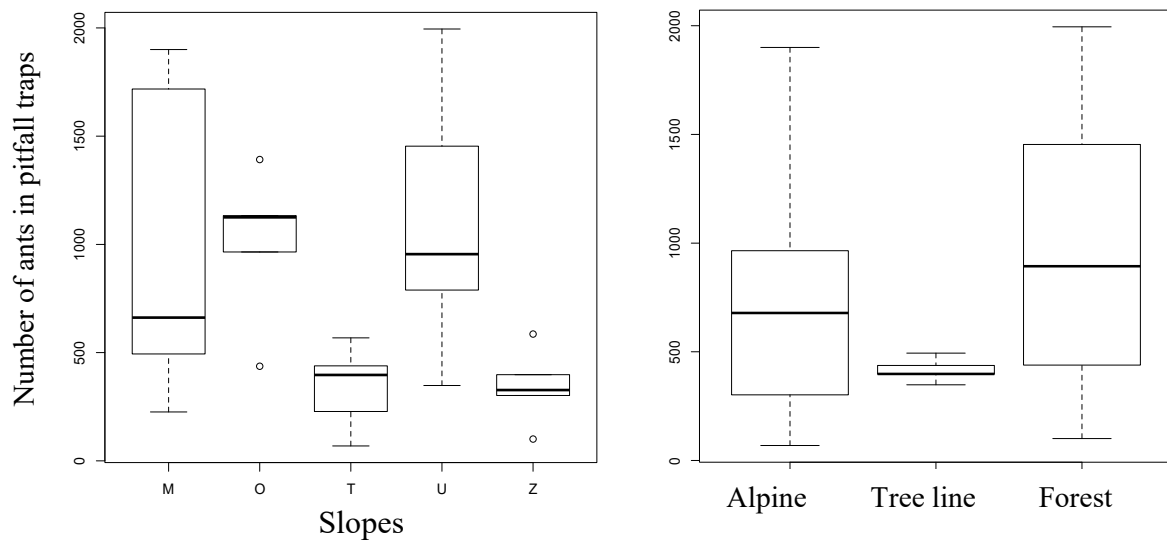
Figure 11. Comparison between the number of worker ants found in pitfall traps between the slopes and the habitats. Slope T and Z in the nature reserve were significantly lower than the sites in the national park. Ant numbers on the tree line were slightly lower and less variable in comparison to the other habitats.

3.3. Community composition analyses

The relative species (pseudo-) abundances were used to compute the distances with regard to ant community composition between all sites to determine (a) if ant communities differed between the three habitats and (b) what might drive such differences. First, unconstrained ordinations (non-metric multidimensional scaling, NMDS) were computed and visualised to get an impression of the community patterns (Fig. 12).

Ant assemblages from the tree line emerge as nested within the alpine sites and were strikingly similar to each other. Subalpine forest ant assemblages, in contrast, showed much higher dispersion, probably due to the high degree of environmental variation that occurred within these sites. Most species centroids clustered together because most of the abundant ant species occurred over the whole altitude gradient. In contrast, species that occurred just in the forest or on and above the tree line were positioned at the edges of the data cloud in reduced ordination space. The two most abundant ant species, *Formica lemani* and *Formica lugubris*, occurred over the whole habitat gradient, but with abundance peaks at opposite positions. They came up at contrasting positions at the margin of the central cluster of ant species in reduced ordination space.
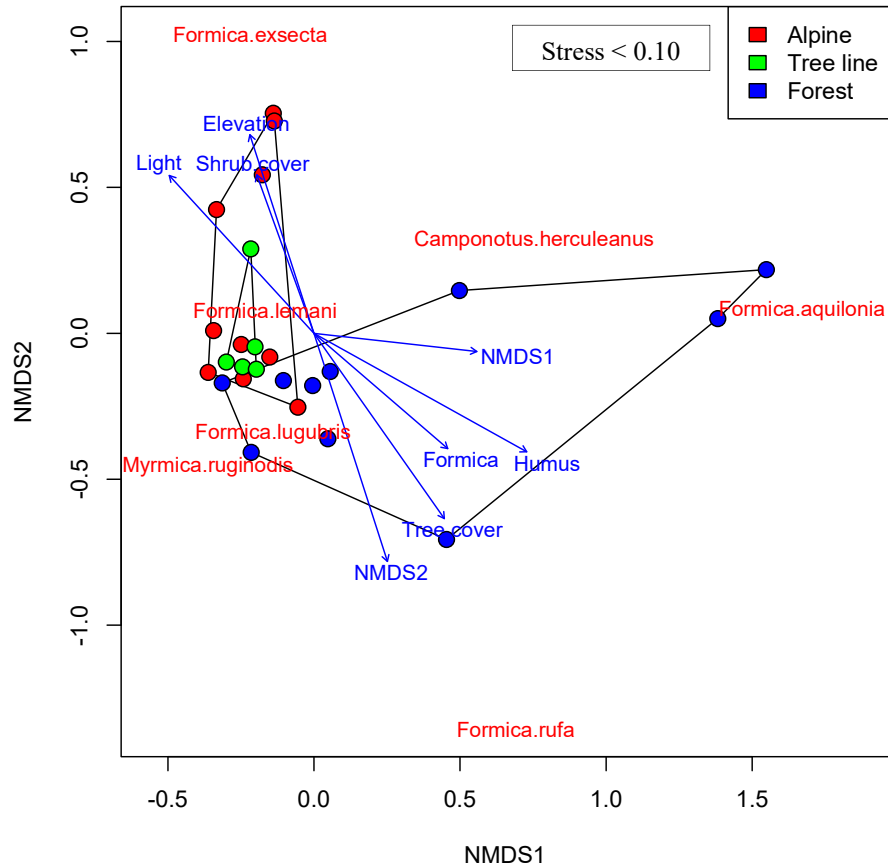
Figure 12. NMDS plot of the ant assemblages across all sampling locations. Colours differentiate the three habitat types. Ant assemblages from tree line sites (green) are clearly nested within the alpine sites (red), but display a lower dispersion in comparison to ant assemblages of the other two habitats. Alpine and forest sites (blue) are clearly clustering apart from another. Centroids of most ant species cluster close together because they occurred along the whole gradient and just some species not plotting on the centroid were displayed. Most of the site variables (superimposed post hoc on the ordination) clearly mirror the habitat gradient (from subalpine forest [lower right] to alpine grassland [upper left]).

To further evaluate differences in the community and their possible drivers, a constrained ordination was performed. Initially, all ant species were used but, after finding out that *Formica* s. str. was strongly associated with the presence of trees ($r = 0.79$) and above the tree line with shrub cover ($r = 0.80$) measured on the sites (also found in this study: Vandegehuchte et al., 2017), these species were excluded. Instead, the total density of mound-building wood ants (scored as their incidence at all baits and in pitfall traps at each site) was used as explanatory variable for all other ant species. Ecologically, this reflects the dominance of wood ants, which are considered to influence the presence and abundance of all other, competitively inferior, ant species. Other factors like the light and humus indicator values extracted from the plant data depicted the elevation gradient rather clearly (see Table 3 with correlation coefficients of variables). Also shrub cover (by *Juniperus communis* and *Rhododendrom ferrugineum*) explained much variance in ant community composition, especially for the tree line sites that had a higher shrub cover than the others. These analyses corroborate that species composition of ant communities across the alpine tree line is shaped by a combination of abiotic factors, but the abundance of dominant mound-building wood ants plays a central role.

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Table 9. Model selection procedure for distance-based redundancy analysis (*capscale* function in R package *vegan*) of ant assemblage composition, with four putative explanatory variables. Nine further factors were tried as explanatory variables, but resulted in far worse model fit and were therefore discarded. AICc defines the Akaike Information criterion corrected, *w* the Akaike weights (Wagenmakers & Farrell, 2004) and R^2 and R^2 adjusted (Legendre et al., 2011) the explained variance.

Model ID	Fixed factors used	Variables omitted	Nr. variables	AICc	Diff. to Model 2	*w*	R^2	R^2 adj.
1	Formica + Light + Humus + Shrub	0	5	24.68	0.79	0.17	41.88	37.79
2	**Formica + Humus + Shrub**	**Light**	**4**	**23.89**	**0.00**	**0.25**	**37.75**	**35.64**
3	Light + Humus + Shrub	Formica	4	26.52	2.63	0.07	32.69	28.49
4	Formica + Light + Shrub	Humus	4	26.74	2.85	0.06	32.23	27.84
5	Formica + Light + Humus	Shrub	4	27.47	3.58	0.04	30.73	25.72
6	Humus + Shrub	Formica + Light	3	25.07	1.18	0.14	29.75	28.55
7	Formica + Shrub	Light + Humus	3	25.67	1.78	0.10	28.43	26.78
8	Formica + Humus	Light + Shrub	3	26.54	2.65	0.07	26.50	24.19
9	Formica	Light + Humus + Shrub	2	27.29	3.40	0.05	18.35	17.91
10	Humus	Formica +Light + Shrub	2	27.34	3.45	0.04	18.23	17.75
11	Shrub	Formica+ Light + Humus	2	29.05	5.16	0.02	13.60	11.91
12	Null Model	ALL	1	31.12	7.23	0.01	0.00	0.00
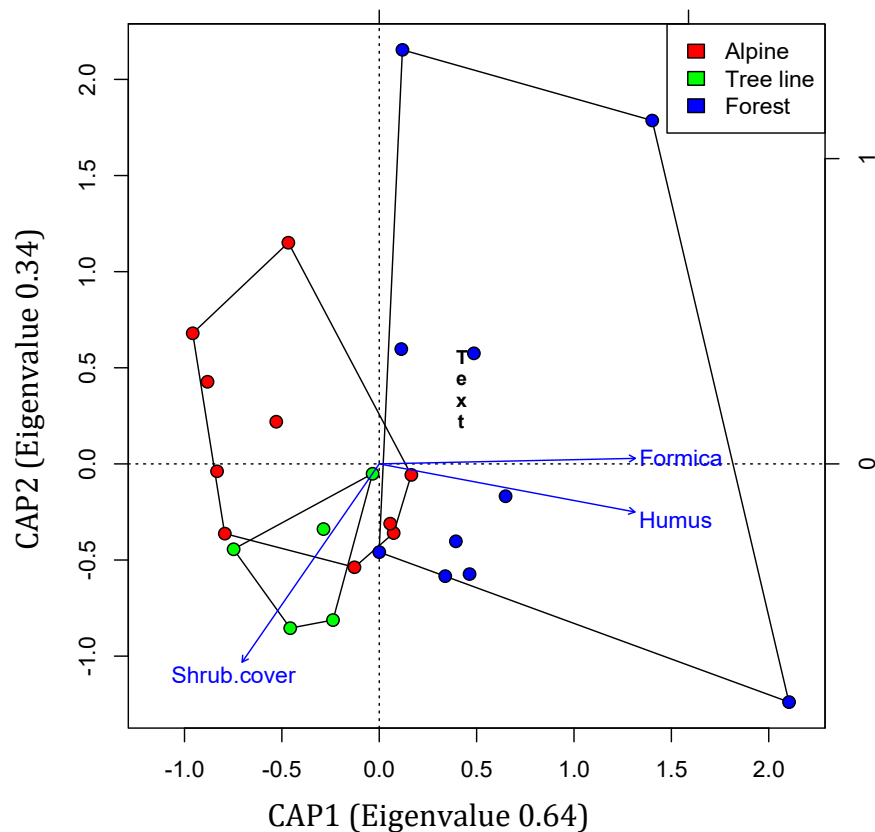
Figure 13. Ordination plot from the capscale routine, showing the influence of the most important explanatory variables (viz. Formica density, Humus indicator value and Shrub cover) on the species composition of ant assemblages. Ant communities from alpine and forest sites cluster apart. Those from tree line sites cluster more with the alpine sites, but display lower dispersion. The Formica s. str. species where here excluded from the ant community matrix and their incidence on baits and in pitfall traps was used as explanatory variable for the remaining fraction of the ant community.

3.4. Functional diversity and community weighted means of traits

For the computation of functional diversity 14 different traits (often split in several sub-traits) were assembled from the literature. For all functional diversity measures and the community weighted means of individual traits an analysis of variance (mixed model ANOVA) was performed to determine whether the changes observed over the ecotone were significant (Fig.14).
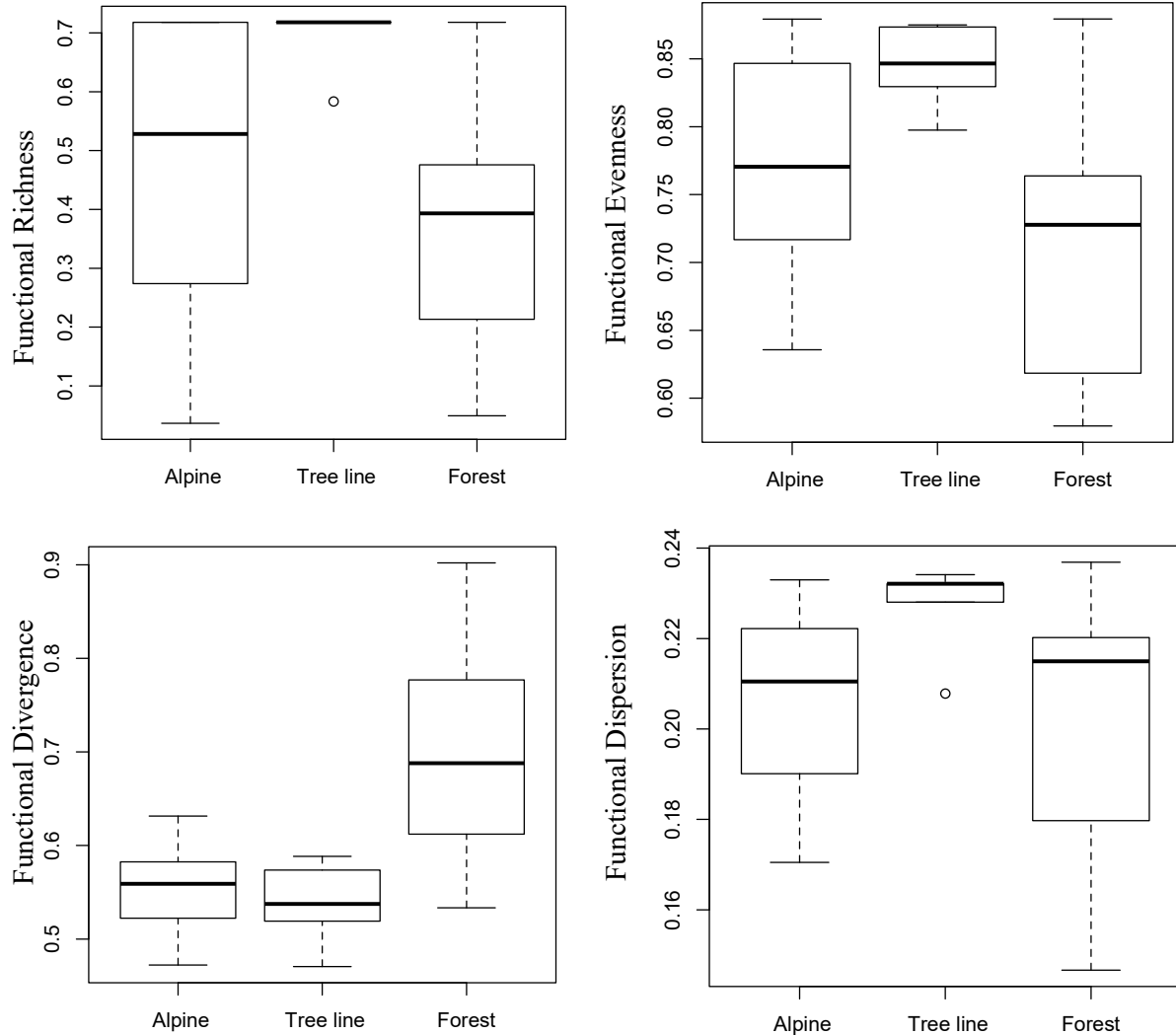

Figure 14. Plots of the four Functional Diversity indices split between habitats. First row depicts the functional richness and evenness and the second row the functional divergence and dispersion.

Table 10. Results of mixed model ANOVAs of various functional diversity indices across the habitats. Slope identity was always modelled as random factor. *: significant at p<0.05.

ANOVA	Degrees of freedom	Sum of Squares	Mean Sum of Squares	F	p
Functional Richness ~ Habitat	2	0.31	0.16	4.64	0.034*
Residuals	18	0.61	0.03		
Functional Evenness ~ Habitat	2	0.06	0.03	4.22	0.031*
Residuals	18	0.14	0.01		
Functional Divergence ~Habitat	2	0.13	0.07	9.73	0.001**
Residuals	18	0.12	0.01		
Functional Dispersion ~Habitat	2	<0.01	<0.01	2.82	0.086
Residuals	18	<0.01	<0.01		

Functional richness differed only between forest (lowest) and tree line habitats (highest). In the alpine grassland an intermediate functional richness was observed (Fig. 14). An analogous pattern was found for functional evenness where also only forest (always lower) and tree line habitats (higher) differed from each other (Fig. 14).

For functional divergence instead, a higher divergence at the forest sites emerged, compared to both alpine and tree line sites. This higher divergence might hint to a character displacement in forest ant assemblages (see below, discussion section). Functional dispersion was not differing significantly (Tab. 10) between the three habitat types, but a similar pattern as for species and functional richness and evenness was clearly visible (Fig. 14).

Community weighted means (CWM) are often used to gain a mechanistic explanation of community differences across environmental contrasts. In view of the rather good ecological knowledge of the European ant fauna these analyses allowed to determine which trait values might change over the ecotone. Only a subset of traits was chosen to be visualised, because directly referring to the already discussed issues of feeding and foraging behaviour as of competition related characters.

Feeding related traits
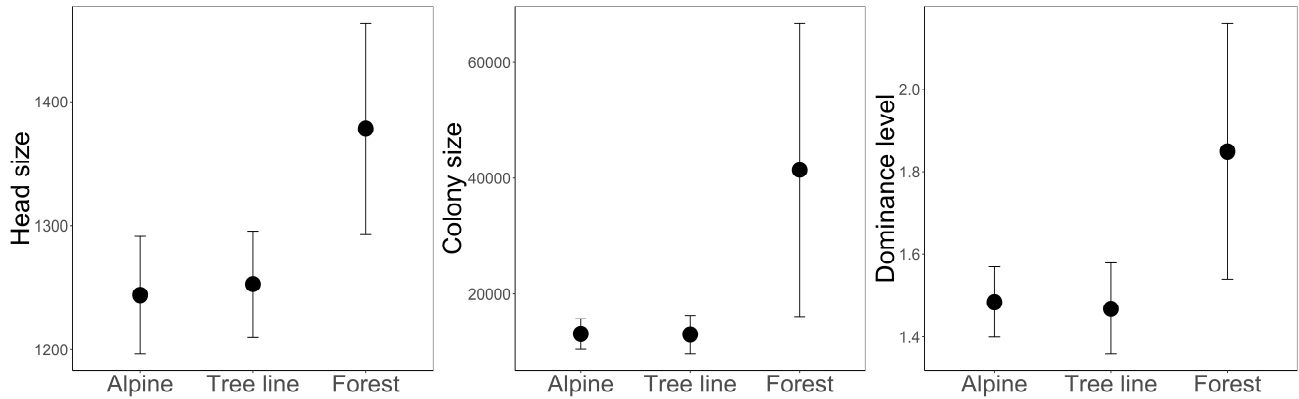

Foraging related traits
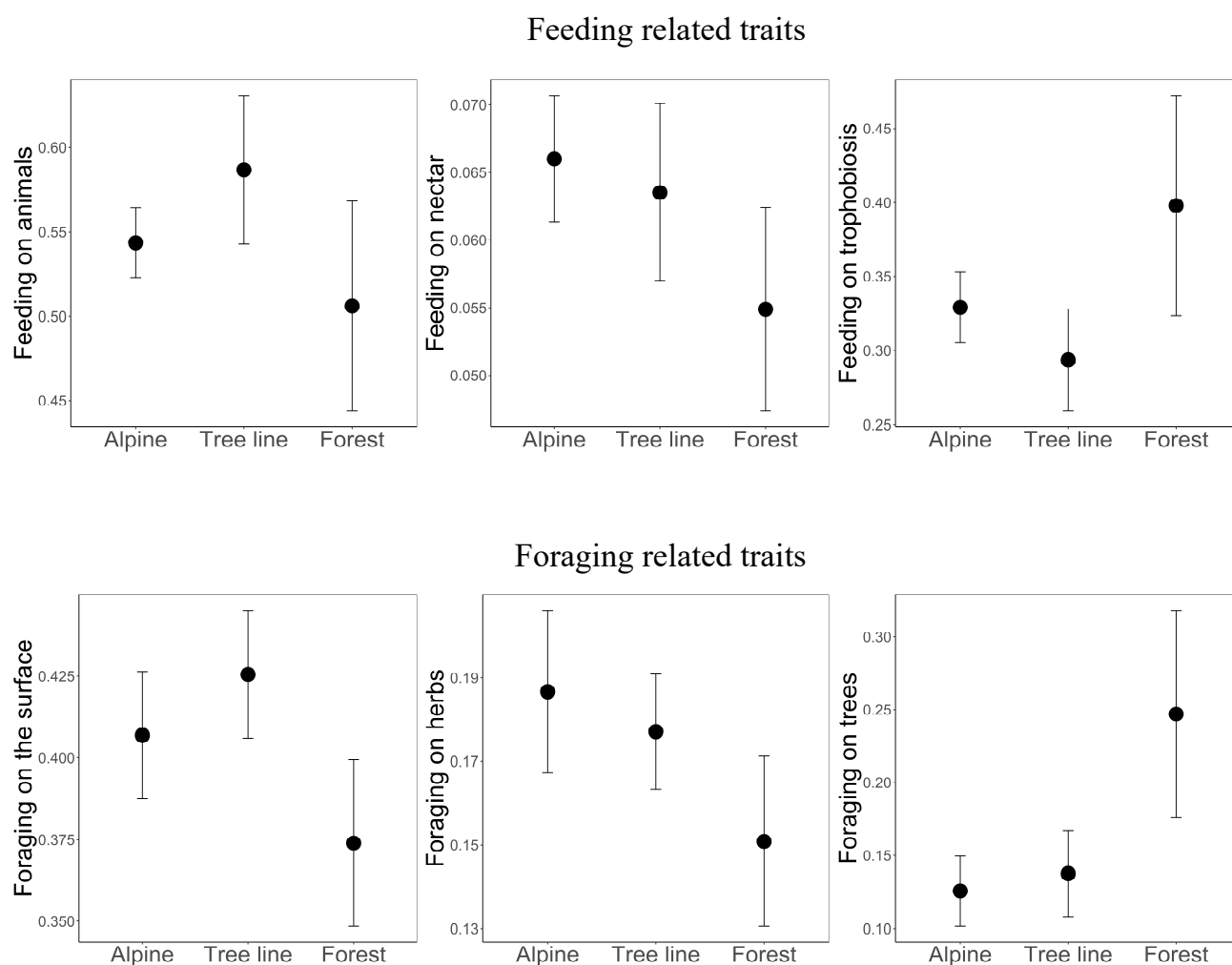
Figure 15. Plots of community weighted means of some important functional traits of ant species. First row: feeding related traits; second row: trait referring to the foraging place; third row: traits referring to the morphology and dominance of ants. For all depicted traits there were significant differences ($p < 0.05$) among the habitats in mixed-model ANOVAs with slope identity as random factor.

Feeding related traits (Fig. 15. first row) showed a reduction in the prevalence of all feeding types in the forest except for trophobiosis, which was more important among forest ant assemblages. A similar pattern can be seen in the strata where foraging takes place (Fig. 15. second row), viz. a reduced propensity to forage in all strata in the forest except for feeding on trees. Traits referring to the morphology of ants (Fig. 15. third row) show an increase in forest, indicating a higher competition pressure that is also apparent from the dominance level of ants (Savolainen et al., 1988). All these patterns are clearly related to the increasing abundance of wood ants in the forest, which are nearly missing above the tree line.

3.5. Results of the baiting experiment

All in all, 1110 Baits were exposed on the 25 sites and 5 slopes. 2406 individual worker ants were recorded at the baits representing 12 different species. The main visitor was *F. lemani*, which was responsible of 76.95% (247 incidences) of the total visitations. Wood ants visited 28.97% (93 incidences) of the visited baits (82% *F. lugubris* and 18% *F. aquilonia*). The Myrmicinae family was on just 10.59% (35 incidences) of the visited bait and Manica rubida visited separately half of these baits (4.98%; 16 incidences). Only 321 baits (28.92%) were ever visited by ants, this value declined from the alpine (37.38%) and the tree line sites (33.70%) to the forest, with just 17.38% of baits attracting some ants (Fig. 16). Visitation incidence also varied between the offered resources. The mixture (of sugar and amino acids) was visited in 31.83% of the cases (99 incidences), followed closely by pure sucrose baits 29.6% (95 incidences). Salt (16.2%; 52 incidences) and glutamine (14.95%; 48 incidences) were visited equally often, while water (7.17%; 23 incidences) and oil (just 1.25%; 4 incidences) were mostly avoided (Fig. 16). On 65 baits more than one species was found, indicating a possible competition for resources and a subsequent resource exploitation.
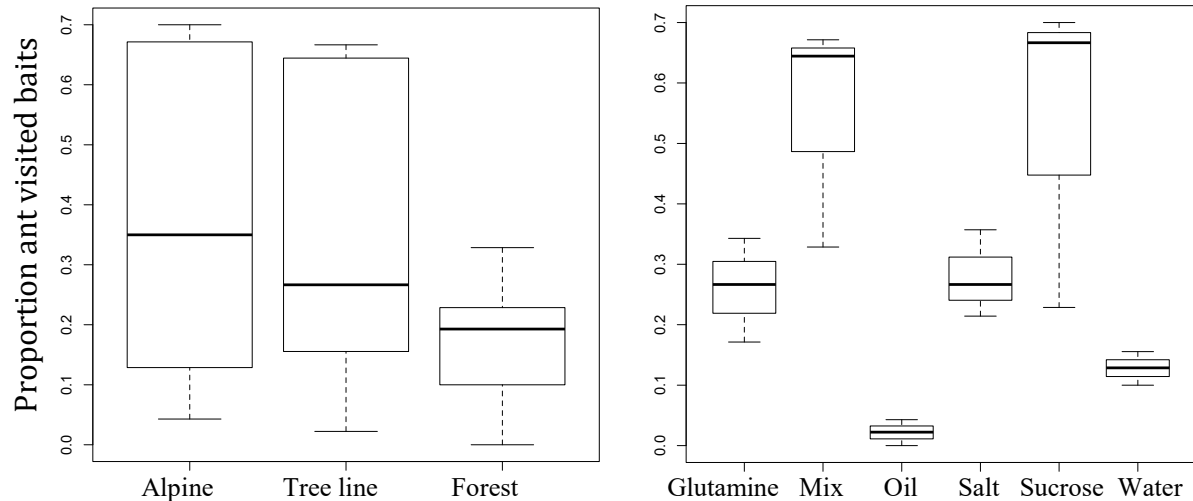
Figure 16. Incidence of ant visits (all ant species combined) segregated according to habitats and bait types. A lower visitation incidence in the forest and of oil and water baits can be seen. The most visited resources were sucrose and a mixture of sucrose and amino acids ('Mix'). Amino acid glutamine and table salt were used similarly in a medium manner.

To evaluate the feeding preferences generalised linear mixed models (GLMM) were computed with just the incidence of any ants at the baits as response variable. Subsequently, a model selection procedure using Akaike information criterion corrected, Akaike weights (w; Wagenmakers & Farrell, 2004) and R^2 (Nakagawa & Schielzeth, 2013) delivered the best model (Tab. 11).

Table 11. Results of model selection for bait visitation. Akaike information criterion corrected, Akaike weights (w; Wagenmakers & Farrell, 2004) and $R^2_{marginal / conditional}$ (Nakagawa & Schielzeth, 2013) were used for model selection.

Model ID	Variables used	Variables omitted	Number variables	AICc	Diff. AICc to best model	w	R2 marginal	R2 conditional
1	Fixed Factors: Bait X Habitat; Random Factors: Slope/Site/Transect + Start Exposition + End Exposition + Activity density + Tree size + Soil temperature + Air temperature	Full Model	11	1084.71	25.14	0.00	46.82	54.63
2	Fixed Factors: Bait X Habitat; Random Factors: Slope/Site/Transect + Start Exposition + End Exposition + Soil Temperature	Activity density + Tree size + Air temperature (each variable made model 2 AICc worse)	8	1067.40	7.83	0.02	46.82	54.63
3	Fixed Factors: Bait X Habitat; Random Factors: Slope/Site/Transect + Start Exposition + End Exposition	2 + Soil Temperature	7	1062.99	3.42	0.15	46.82	54.63
4	**Fixed Factors: Bait X Habitat; Random Factors: Slope/Site/Transect +End Exposition**	**3 + Start Exposition**	**6**	**1059.57**	**0.00**	**0.83**	**46.49**	**54.68**
5	Fixed Factors: Bait; Random Factors: Slope/Site/Transect + Start Exposition + End Exposition	3 + End exposition	5	1079.66	20.09	0.00	45.79	52.9
6	Fixed Factors: Habitat; Random Factors: Slope/Site/Transect + Start Exposition + End Exposition	3 + Bait	5	1287.76	228.19	0.00	7.46	14.59
7	Fixed Factors: Bait X Habitat; Random Factors: Slope/Site/Transect	3 + Habitat	5	1081.96	22.39	0.00	33.57	49.55
8	Null Model: No Fixed Factors Random Factors: Slope/Site/Transect + End Exposition	3 + Bait + Habitat	4	1306.80	247.23	0.00	0.00	14.78

According to the best model, acceptance of baits was mostly affected by the resource offered. Also habitat scored significantly, meaning a change in resource acceptance took place between the habitats. The significant interaction between habitat and sucrose clearly shows a reduction in the usage of sugar usage in the forest (Tab. 12; Fig. 17).

Table 12. GLMM results for the best model (#4 in Table 11). *: significant at p<0.05; **: significant at p<0.01; ***: significant at p<0.001.

Generalised linear mixed model	Estimate	Std. Error	z value	p
(Intercept) / Glutamine	-0.94	0.24	-3.89	< 0.001 ***
Mix	1.37	0.25	5.54	< 0.001 ***
Lipids	-3.47	0.89	-3.90	< 0.001 ***
Salt	0.14	0.25	0.55	0.581
Sucrose	1.26	0.25	5.04	< 0.001 ***
Water	-0.94	0.29	-3.21	0.001 **
Habitat	0.63	0.23	2.72	0.006 *
Mix X Habitat	0.29	0.28	1.02	0.308
Oil X Habitat	0.93	0.98	0.95	0.343
Salt X Habitat	-0.11	0.29	-0.38	0.707
Sucrose X Habitat	0.63	0.29	2.15	0.032 *
Water X Habitat	-0.38	0.34	-1.12	0.265
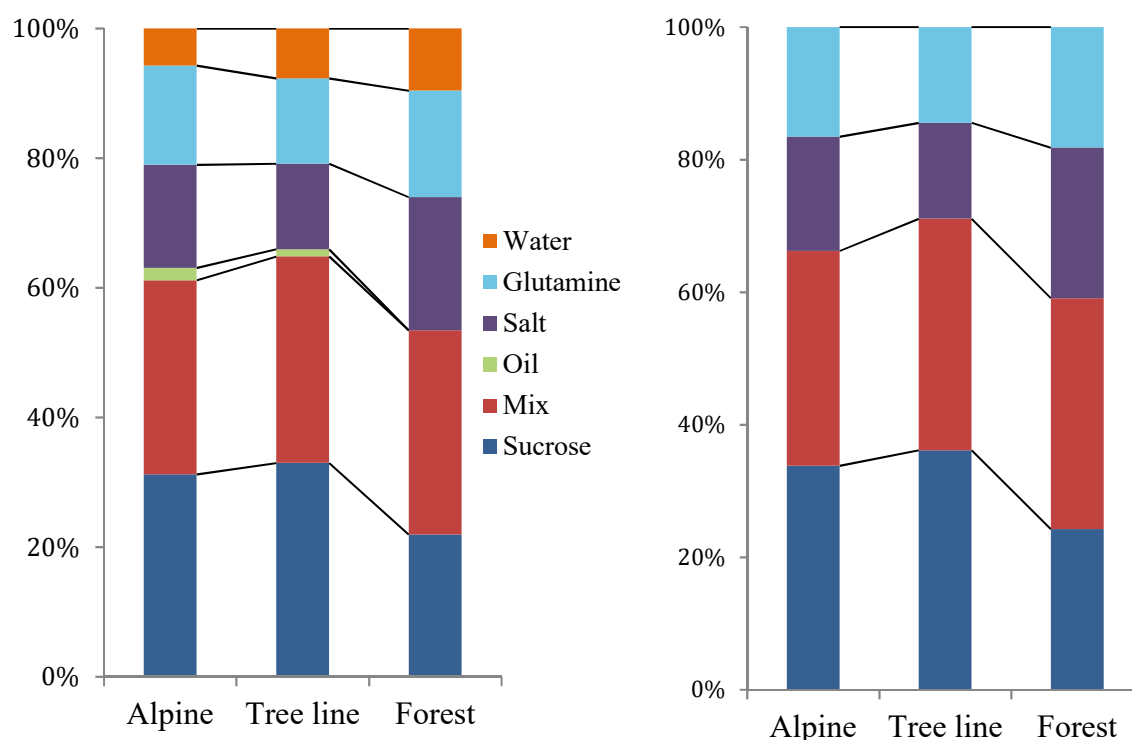
Figure 17. Bait visitation of all ants across the three habitats. A reduction in the usage of sucrose towards the forest can be clearly seen. Further a slight increase in the usages of both salt and proteins in the forest is also visible. Oil and water were used very few times, but the first just in the alpine site and the second increases in the forest. In the right panel, water and oil were omitted to allow a clear comparison between the more intensely used resources and their changes over the ecotone.
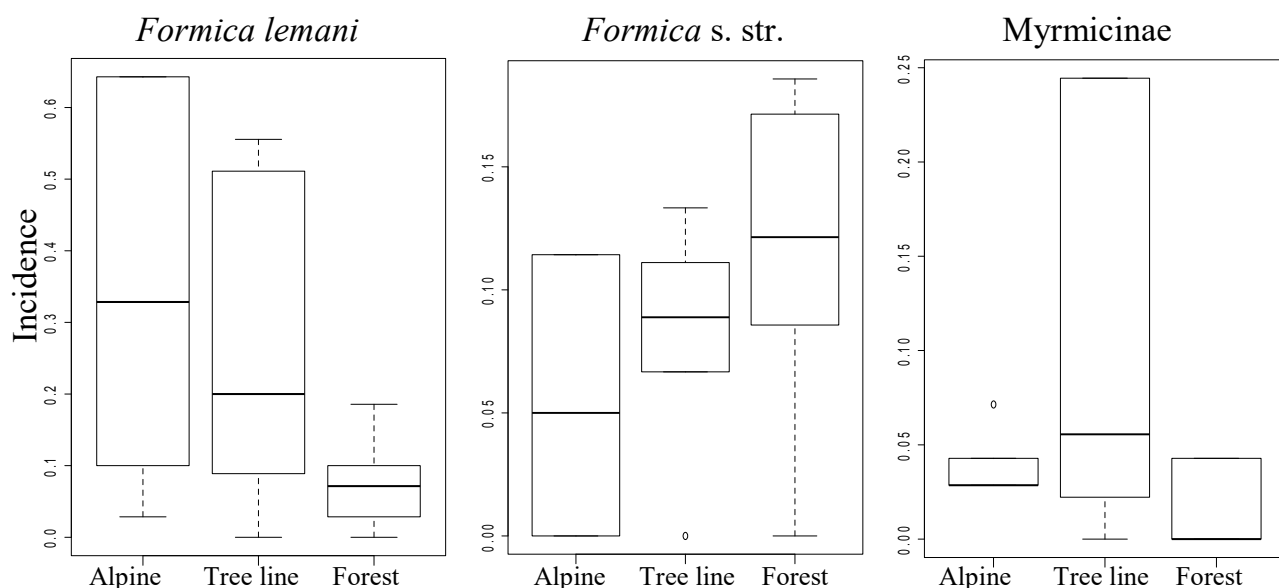
Figure 18. Incidence of bait visitation by particular ant species groups across habitats. *Formica lemani* with 77% of all ant visits, subgenus *Formica* with 29% visited baits and Myrmicinae accounting for just 15% of all visits at the baits. *F. lemani* visitation decreased in forest, while *Formica* s. str. concomitantly increased. Myrmicinae ants instead showed an increased presence on baits at the tree line, where the incidence of the two groups within *Formica* was lower.
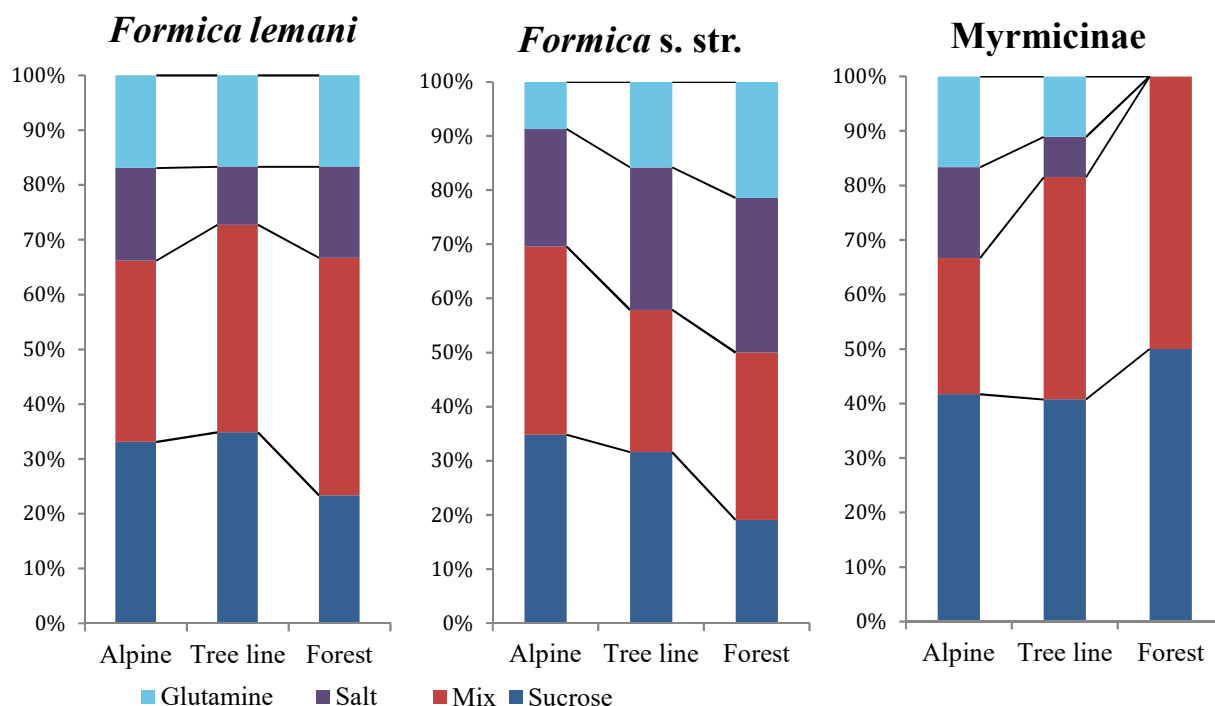
Figure 19. Feeding preferences of *Formica lemani*, *Formica* s. str. and all species of the Myrmicinae turning up on baits. In the first two, patterns are similar with a decrease in the usage of sucrose and an increase in visitation of amino acids and salt. The pattern among Myrmicinae is the opposite, showing an increased usage of sugar in the forest.

The visitation intensity and preference was also computed separately for *Formica lemani*, *Formica s. str* and all the Myrmicinae species together. Because of the very low visitation of the

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last group only a graphical incidence is informative. The result of the GLMM about the single species or groups are resulted in a similar pattern as the overall result (Tab. A in Appendix).

3.6. Stable isotope analysis

Stable C and N isotope analysis showed, as expected, a clear differentiation between plants, herbivores, and ants (Tab. 14). Also the two ant species *Formica lemani* and *Formica s. str*. showed a clear segregation among each other.
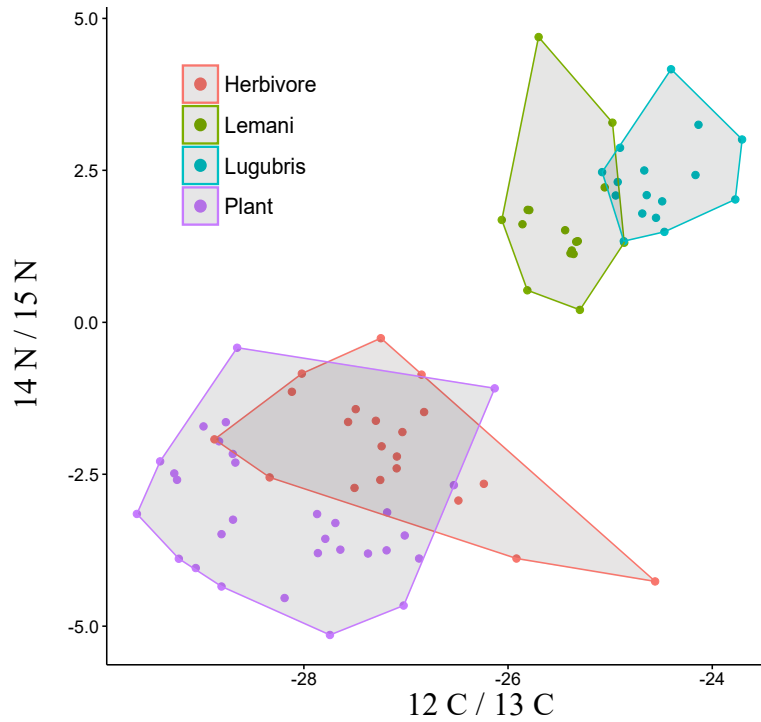
Figure 20. Scatterplot of all analysed samples grouped as Plants (woody and herbaceous plants combined), herbivores (grasshoppers, Lepidoptera larvae and Sternorrhyncha combined) and the two main ant players along the gradient, viz. *Formica lema
ni* and *Formica* s. str (mainly *F. lugubris*, one sample was *F. aquilonia*).

Both heavier isotopes, carbon and nitrogen, seem to accumulate in the food chain from the lowest score by plants to the highest in *Formica* s. str.

Table 13. Mixed model PERMANOVA results for both isotope proportions combined, based on Euclidian distances. Slope identity was modelled as random factor and 9999 permutations were used. *: significant at p<0.05.***: significant at p<0.001.

Source	df	SS	MS	Pseudo-F	p (MC)
Sample	3	611.11	203.70	193.55	< 0.001 ***
Habitat	2	12.44	6.22	5.91	< 0.001 ***
Slope ID	4	22.33	5.58	5.30	< 0.001 ***
Sample X Habitat	6	13.02	2.17	2.06	0.033 *
Residuals	66	69.46	1.05		
Total	81	741.13			

Table 14. Mixed model PERMANOVA results of pairwise differences between plants, herbivores and the two ant species, based on Euclidean distances computed with both C and N isotopes showing group differences. Slope identity was modelled as random factor. ***: significant at p<0.001.

Groups	t	p (MC)
Plant ~ Herbivore	4.28	< 0.001 ***
Plant ~ Lemani	15.95	< 0.001 ***
Plant ~ Lugubris	20.10	< 0.001 ***
Herbivore ~ Lemani	11.81	< 0.001 ***
Herbivore ~ Lugubris	16.71	< 0.001 ***
Lemani ~ Lugubris	4.68	< 0.001 ***

The hypothesis behind this analysis was that in forest, a lower position of the ant community in the food web was expected due to the higher abundance of trophobiotic homopterans that provide a nearly direct usage of plant produced compounds by the ants. To evaluate this possibility, we performed first a PERMANOVA comparing the habitats with regard to accumulation of the heavier nitrogen isotope and found that for all analysed groups except the ants there was a significant difference (Tab. 15; Fig. 21).

Since the isotope signature of nitrogen was significantly higher in the forest in both plants and herbivores, the whole food web can be considered to be based on a higher fraction of the heavier nitrogen isotope. Following this consideration the nitrogen signature of the ants occurring in the forest should also be higher as the signature of the ants occurring over on and over the tree line if the tropic position (and so the feeding habits) would not change over the gradient. But the nitrogen isotope signature of the forest ants is equal to the signature of the ants from the other locations (for both species) indicating that there is a significant lower score on the forest ant isotope fraction. This lower score is considered to be determined by the consistent contribution of plant derived carbohydrates and nitrogen that ants retrieve through trophobiosis association that are present in high numbers in the forest.

Table 15. Mixed model PERMANOVA result of pairwise differences between alpine, tree line and forest sites, with all samples analysed. Slope identity was modelled as random factor.
*: significant at p<0.05. ***: significant at p<0.001.

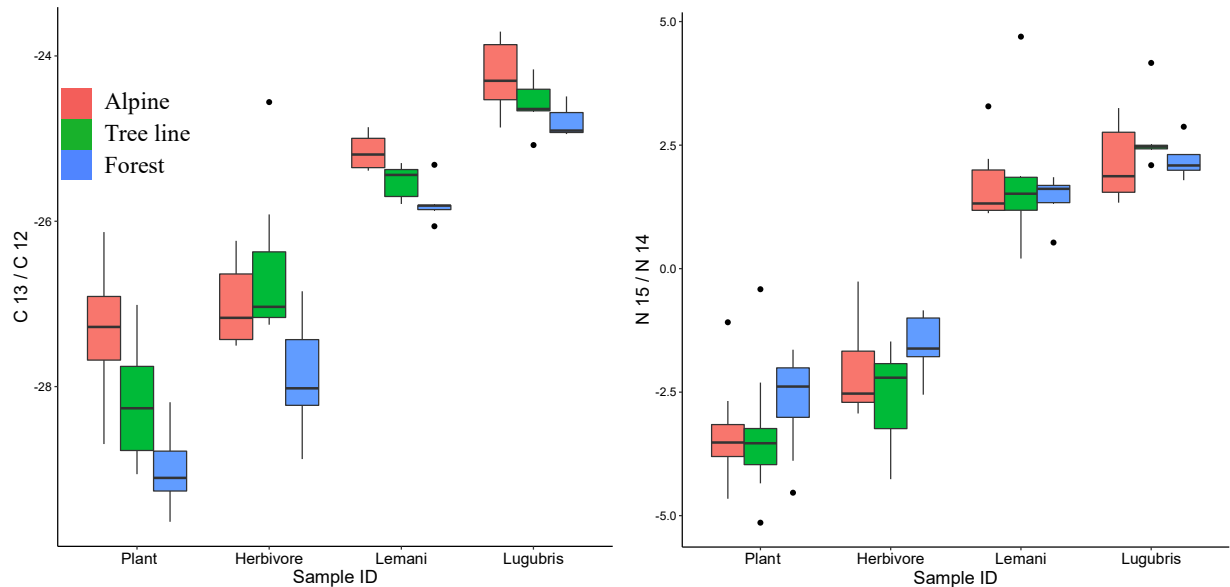| Groups	t	p (MC)
Alpine ~ Tree line	0.95	0.389
Alpine ~ Forest	3.71	< 0.001 ***
Tree line ~ Forest	2.15	0.017 *

Figure 21. Boxplot of both isotope (C and N) signatures of plants, herbivores and ant species, partitioned among sampling habitat in the gradient. A reduced proportion of the heavier isotope of carbon can be seen in all sample groups in forest. For the nitrogen isotope instead we see a higher score in forest for both plants and herbivores, but no differences in case of both ant species.

3.7. Result from sampling of other ant-related taxa

3.7.1. Homopterans and trophobiotic associations

The sampling of plant lice delivered highly variable data. At some sites or slopes I observed lots of interactions, while at others there were none. Probably the sampling on just one day per site was not exhaustive enough to quantify clearly the amount of the trophobiotic interaction. Hence, data structure precluded a proper statistical analysis, and accordingly a descriptive approach was chosen (Tab. C in Appendix).

Formica lemani and *Formica lugubris* were responsible for nearly all the interactions. On the other hand, the genus *Cinara* was the predominant partner in these associations. The only exception was *Manica rubida* found interacting with a yet undetermined representative of Coccoidea on *Juniperus communis var. saxatilis*.

The interaction network of ants, plant lice and plants on which these were present is shown in Figure 22. A tentative attempt to quantify the strength of the trophobiotic interactions is presented in Table 16. The number of visited trees is given for those trees where an ant visitation was observed but the homopteran aggregations where located too high up in the trees to be reached. It can be clearly seen that *Formica* s. str. was the dominant partner in these associations and increased in dominance at lower elevations, e.g. in forest. *Formica lemani* had a higher visitation of trophobionts in the alpine setting compared to *Formica* s. str. and a similar one at the tree line. Interestingly no interaction of *Formica lemani* with aphids was detected in forest. The dominant interaction in the alpine setting was of *Cinara juniperi* on *Juniperus communis var. saxatilis* with both *F. lemani* (also on single individuals) and *F. lugubris* (only if the aggregation was bigger; >2 aphids). Further treelets (*Pinus cembra* and *Picea abies*) if present were often highly colonised by *Cinara* (*C. cembrae* for the first and *C. piceicola* for the second) and visited by *F. lugubris* and rarely by *F. lemani*. The abundance of *Formica lugubris* above the tree line was found to highly

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correlate (r = 0.80) with the cover of dwarf shrubs (*Juniperus communis* and *Rhododendron ferrugineum*, but no plant lice were found on the latter plant species).

Further a Coccoidea species (undetermined) on *Juniperus communis var. saxartilis* was found to interact with *F. lemani* and *Manica rubida*. This scale insect was found on twigs of *Juniperus communis* lying on the ground and being covered by organic materials. The occurrence of this species was by coincidence detected only on the last slope, but then found on all remaining sites above the tree line. On the tree line and in the forest the interaction with plant lice was much more common and most trees present on transects were visited by ants. On the tree line 74.72% of the trees had ant visitation on them, in the forest just 52.29% of the trees were visited.

Table 16. Numbers and proportions of trophobiotic interactions, split between *Formica lemani* and *Formica* s. str.. Also the visited trees were noted where ant visitation occurred and trophobiotic interaction can be expected to occur, but these were located too far up to be reached.

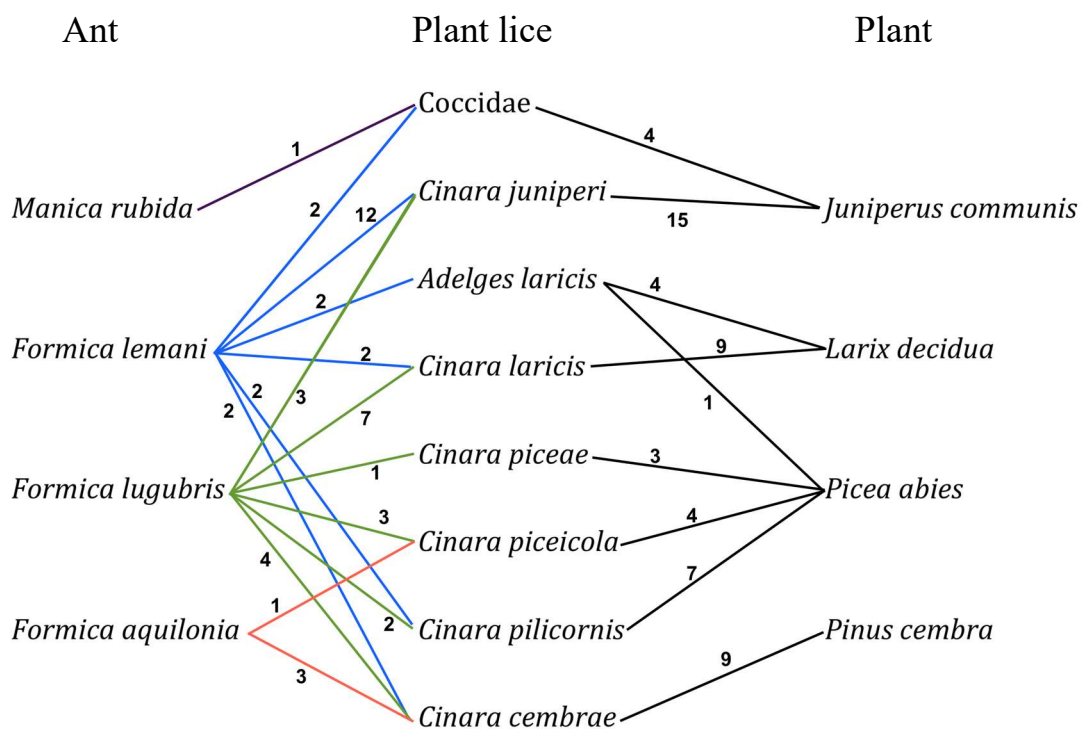| Number transekts	Habitat	% visited colonies by *Formica lemani*	Trees visited by *Formica lemani*	% visited colonies by *Formica* s. str.	Trees visited by *Formica* s. str.	Plant lice species
30	Alpine	37 %	0	27 %	4	6
15	Tree line	53 %	21	53 %	28	8
30	Forest	0 %	0	23 %	61	7

Figure 22. Interaction network between ants, plant lice, and plants. The numbers above the lines indicate the number of transects (three per site) on which these interactions were found. Coloured lines define to which ant species the interaction belongs.

3.7.2. Spiders and ground beetles

249 individuals of ground active hunting spiders and 222 individuals of ground beetles were found in the pitfall traps. The effect of wood ants on spiders and ground beetles was tested first with a simple linear regression between the number of individuals and the density of *Formica s. str.*. A clear negative correlation was found only for spiders ($r = -0.48$) but not for ground beetles ($r = 0.19$; Fig. 23). A linear mixed model delivered a significant effect of the abundance of *Formica* s. str. on the numbers of spiders (likelihood ratio test, $p < 0.001$), but not for the number of ground beetles (Likelihood ratio test, $p = 0.68$; Tab. 17; Fig. 23).

Table 17. Results of Linear mixed models (LMM) relating the number of spiders and ground beetles found in the pitfall traps to the abundance of *Formica* s. str.. Slope identity was modelled as random factor. ***: significant at p<0.001.

Linear Mixed Model	Estimated	Standard Error	*t* value	*p*
Intercept	4.25	0.66	6.45	< 0.001 ***
Number of Spiders ~ Formica	- 2.77	0.67	- 4.12	< 0.001 ***
Intercept	22.53	0.55	4.58	< 0.001 ***
Number of Ground Beetles ~ Formica	0.35	0.87	0.40	0.68
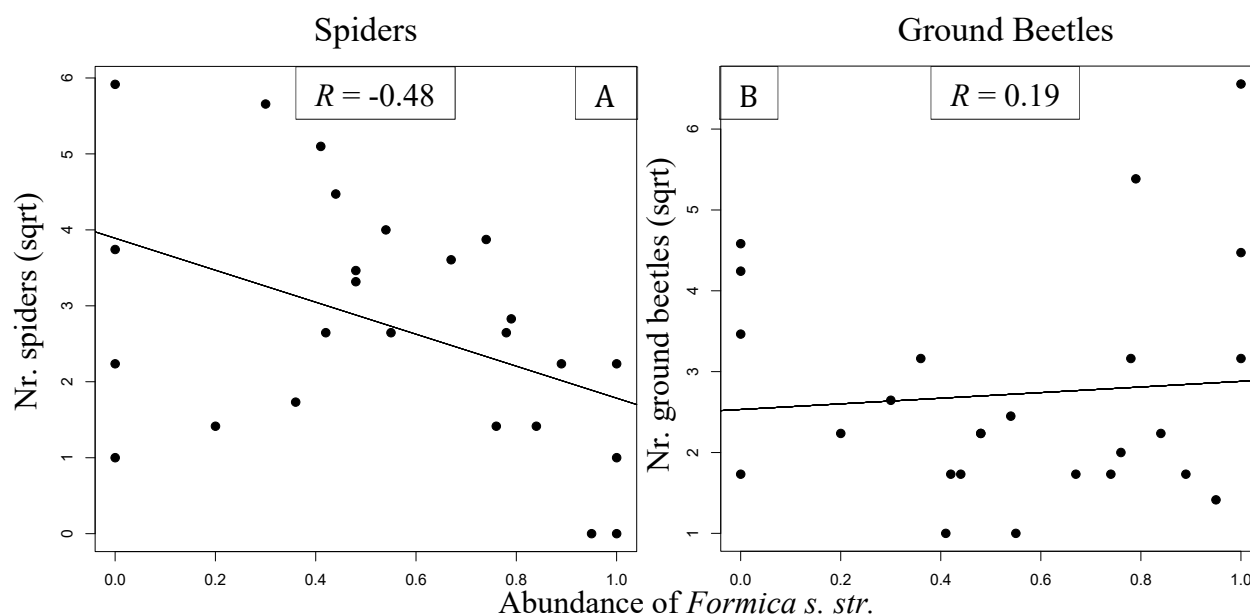
Figure 23. Ordinary linear regression between the number of individuals (square root transformed) of spiders (A) and ground beetles (B) relative to the density of wood ants (measured as the % of incidence on all baits and in all pitfall traps exposed on a site).

Altogether, my samples comprised 12 species of ground beetles (Tab. B in Appendix). With this information an unconstrained ordination was computed showing that the geographical effect on ground beetle species composition was highest for these predominantly wingless beetles, whereas *Formcia* abundance as well as habitat played but minor roles. The geographic effect is due to a rather wide and low valley (Vinschgau Valley) dividing the two parks from one another.
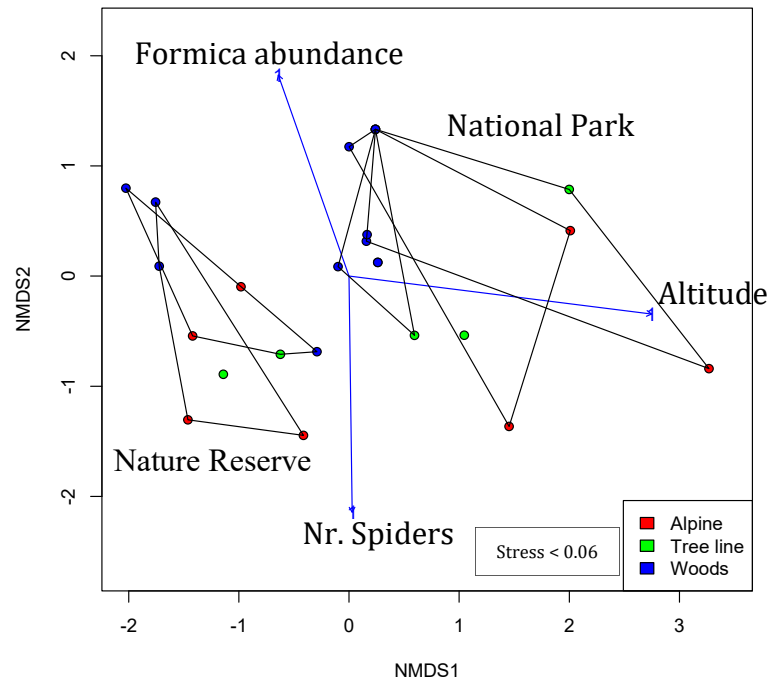
Figure 24. An NMDS ordination of the ground beetle assemblages shows a clear geographical segregation (first axis) and a less prominent habitat and *Formica* s. str. driven pattern (partially explained by the second axis).

4. Discussion

4.1. Ant community composition

The forces shaping animal communities are varied and can be summed up as biotic and abiotic factors. The abiotic forces form an important part of the habitat filter. In the case of an alpine habitat the most relevant physical filter is represented by temperature (Sanders et al., 2007; Reymond et al., 2013). For central-place foragers that live together in large societies like ants, the major biotic force is instead often considered to be competition between ants themselves (Hölldobler & Wilson, 1990). However, the role of competition in shaping ant communities has recently be challenged since it is hard to obtain unequivocal data about its real effects. Sometimes competition is even criticised not to be a "hallmark" of ant ecology at all (Cerda et al., 2013). Nevertheless, several studies have concordantly found an inverse relation between the intensity of competition and altitude (Cerda et al., 2013 and citations therein). Hence, with a reduction in biotic constraints, abiotic filters are considered to act more severely with increasing altitude (Machac et al., 2011; Reymond et al., 2013). This change of importance of constraints was expected to shape the ant communities over the rather narrow gradient investigated here, from the subalpine forest to the alpine grassland. This expectation was largely confirmed by the observed changes in community composition over the ecotone. A high proportion of mound-building wood ants were found in the forest (Tab. 3) and their dominance influenced the presence and abundance of the other ant species (Fig. 13; Tab. 9), an already known effect by this dominant ant subgenus (Savolainen et al., 1988; Johansson & Gibb, 2016). Also ground active spiders reacted to the abundance of wood ants with a reduction in the number of individuals (Fig. 23) as found in other studies (e.g. Halaj et al., 1997; Johansson & Gibb, 2016). Ground beetles instead did not show any pattern in relation to the presence of *Formica* s. str. (Fig. 23).

Cerda et al. (2013) called into question the conclusion that a lack of co-occurrence can be interpreted as a sign of competition between ants. Along a similar line, Fowler et al. (2014) found, in an interesting study on ant communities in a temperate forest in North America, that resource availability is shaping the ant community composition (a niche filtering effect) rather than competition among the species (i.e. species occurrences could not be attributed to niche partitioning). This conclusion, if applied to the present study, this could indicate that the presence of honeydew-producing homopterans (and thus a higher availability of carbohydrates in the forest), or of nesting places (reduced availability of stones under which most alpine ant species found their nests; Seifert 2017) might influence the community composition more than the supposed competition. Another possible interpretation is to consider a break-down of ant community composition along the present gradient as found in Canada when bogs and adjacent forest were compared (Gotelli & Ellison, 2002). In this study the ant community changed in the way that in the stressful setting of bogs the community was more randomly assembled than in the forest. This break-down of the community composition, driven by an increased severity of habitat filtering in the bog, allows a comparison with the here investigated gradient where most ant species occurred over the whole gradient, and mainly changed in their local abundance.

In the present study ant community composition and its possible drivers were analysed with a redundancy analysis. The abundance of *Formica* s. str. explained significantly the presence and abundance of other ant species (Tab. 9). While this approach is not the optimal way to evaluate if competition is really the force shaping the community composition in the forest and in this study competition was not targeted directly, this observation leaves good reason to infer that competition plays a central role. Further investigations addressing specifically community assembly rules in the alpine setting or along elevation gradients, using for example null models for given communities or analysing direct competition interactions, could shed more light on this very interesting and poorly addressed topic: What really drives ant community composition over an elevation gradient? Is competition (niche partitioning) or rather resource availability (niche filtering) the more important process?

The ant assemblages living directly at the ecotone sites scored more similarly to those of the alpine sites in several aspects, e.g. community composition, feeding preferences and limitations, isotope fractions and trophic position, several community-weighted means and functional diversity indices. This was quite surprising because the presence of trees was considered to be a central aspect in determining the abundance of the dominant subgenus *Formica* s. str. and thereby indirectly shaping the presence and abundance of all other ant species. Also the occurrence of more trophobiotic aphids on trees directly at the timberline would have suggested the opposite result (Tab. 16). Probably, an edge effect shaped the relative densities of the species more strongly than the presence of trees. Edge effects were found to negatively impact arthropods that are specialist for undisturbed habitats (Duelli et al., 1990), as probably happened with the wood ants (which themselves influenced the other species presence) that tend to occur in the forest interior and already showed a decreased presence on the tree line, where a more alpine character is displayed by the ant community.

In the constrained ordination, the tree line sites scored differently also from the alpine sites (with which they clearly clustered in the unconstrained ordination). This tendency seems in part to be explained by the higher coverage of shrubs (*Juniperus communis* and *Rhododendron ferrugineum*), often present on the tree line sites (Fig. 13), influencing the more frequent occurrence of some species of the Myrmicinae subfamily.

Remarkably, ant assemblages from the tree line sites had a limited variance in several analyses in comparison to the sites situated within either forest or grassland habitats, despite all five tree line sites were located on different mountain slopes. This outcome could be influenced by the smaller sample size, just five tree line sites compared to 10 sites in the other habitats. Still, this similarity is quite striking and might indicate a characteristic of the ecotone, harbouring a similar ant community. In both constrained (Fig. 13) and unconstrained (Fig. 12) ordinations the tree line sites scored more similar to each other. Also with regard to species richness (Fig. 5), number of ant individuals (Fig. 11) and all functional diversity indices (Fig. 14) the site-to-site similarity amongst tree line samples was higher than within the two neighbouring habitats. The higher variance of the ant assemblages at sites within the habitats could be determined by differing factors like canopy closure, the age of the trees, the exposition on ridges and the occurrence of small sunny openings in the forest and changing shrub (and dwarf shrub) and stone cover in the alpine setting that might have influenced the ant community composition.

4.2. Species richness

The number of species found on the sites were more or less expected when compared to other ant studies in the alpine region (e.g. Glaser 2006; Spotti et al., 2015), but on few sites in the alpine setting (were up to eight species were detected) this number was still quite high.

The tree line ecotone is a classical example of a natural edge between two very different ecosystems and often higher species richness was found on forest-grassland edges than in either forest or grassland alone (e.g. Risser, 1995 and citations therein). Also in the present study ant species richness was found to be significantly higher on the ecotone. This pattern was expected, but less expected was the reason in this particular case. It turned out that a higher incidence of species of the Myrmicinae subfamily (five of the seven species of this subfamily occurred more often on the ecotone than in the other two habitats; Fig. 6) drove this pattern, instead of a "classical" mixing between two communities belonging to the contiguous habitats. Only one species was found just in the grassland and it occurred until the tree line, *Formica exsecta* (Seifert 2007). All ant species occurring just in the forest (five species) did not reach the tree line even once, although it is possible they did so in the vicinity of the sample locations.

The species of the Myrmicinae subfamily observed in the alpine environment are all known to be rather subordinate in local ant dominance hierarchies (Savolainen et al., 1988). So their more frequent occurrence at the tree line could be a sign that interspecific competition (mainly driven by *Formica* s. str.; Stockan & Robinson, 2016) is less prominent on the ecotone itself. In line with that inference, the lower individual numbers of ants in the pitfall traps on the ecotone (although not significant; Fig. 11; Tab. 8) could indicate that a less densely packed ant community allows for the occurrence of more (subordinate) species. On the other hand, abiotic habitat filters are probably less prominent at the tree line than in the open alpine setting, because the presence of trees might buffer temperature extremes or even increase the local mean temperature (Lee et al., 2011; Li et al., 2015). Both mean air and soil temperature measured just during the sampling time did not differ between the habitats (Table 1), but the temperature conditions that limit ant distributions probably occurring in the cold season. A possible effect also contributing to the higher ant species richness at the ecotone is the higher primary production (in comparison to a lower one in the alpine setting). Primary production was found to increase the diversity of ant species in other systems (Kaspari et al., 2003).

My results also agree with predictions derived from the dominance-impoverishment rule as formulated by Hölldobler & Wilson (1990). According to this concept, with an increased presence of dominant ants (both in terms of species and of individuals) occurring at a location, fewer other ant species should be able to co-occur. This theory was mainly developed for ant assemblages in temperate regions and has often been refuted by studies in the tropics (e.g. Andersen, 1992; Lach et al., 2010; Ellwood et al., 2016). However, the species richness pattern over the tree line ecotone observed here rather precisely follows this concept. The dominant *Formica* s. str. group that occurs in high number in the forest (Fig. 18), decreases in prevalence directly at the ecotone, thereby enabling more species to persist locally. In the alpine setting a lack of habitat structure provided by trees (and lower primary production) together with a harsher climate could limit the occurrence of more species. Overall, beta species diversity was higher in the forest than in the alpine zone or directly at the tree line ecotone (Fig. 9) as a probable effect of more structured habitat and higher primary production.

4.3. Functional diversity and trait variation

Functional diversity is often seen as a promising way to obtain a better mechanistic understanding of a community, how it is influenced by the environment and how it influences itself the surrounding (e.g. Hooper et al., 2002). The potential of this method increases with the biological knowledge of the species involved. Given the very good knowledge of the central and northern European ant fauna this approach will probably unfold in the near future, even though few attempts have thus far been published to address functional diversity of ant assemblages in a rigorous manner. The publication of Seifert (2017) has made much information about the life history of common ants accessible. Two rather recent studies about ant communities on elevation gradients in the northern hemisphere analysed ant diversity, one focusing on phylogenetic diversity (Reymond et al., 2013) and the other on functional diversity (Machac et al., 2011). Both studies agreed in finding a general decrease in several diversity measures and explained this pattern as a result of harsher abiotic filters and reduced biotic pressures with increasing altitude.

The general pattern of the functional diversity indices in the present study was different: with higher scores directly at the tree line and lower scores, but similar to each other, in forest as well as alpine grassland. The only exception was functional divergence, which displayed the highest score in the forest. Functional richness was significantly higher at the tree line, similar to species richness, and both these community attributes are known to correlate with each other (Hooper et al., 2002). Nevertheless, this score depicted a greater functional breadth of the tree line ant community. Functional evenness, as an abundance-weighted index (for the abundance weighting the relative (pseudo-) abundance of species was used), depicts the evenness of both trait-based distance of the species and the abundance evenness of species (Villéger et al., 2008). The higher score observed at the tree line is probably due to a higher abundance of the Myrmicinae (under-represented in both other habitats), that increases the functional evenness of the community, rendering the ant community not only more diverse, but also more balanced. Functional dispersion revealed a similar pattern as functional richness and evenness, but without changing in a significant way. Still its higher score on the tree line means that ant assemblages there are functionally more diverse, since this index is measured by taking the mean distance between the species which themselves are abundance-weighted (Laliberté & Legendre, 2010).

Functional divergence was the only index scoring differently, with significantly higher values in the forest and lower values at the tree line and alpine sites. This result clearly indicates a higher

character displacement among ant species co-occurring in the forest. Character displacement is considered a clear sign of competition, because it enables the occupation of differing niches, thereby mitigating direct competition between species (Dayan & Simberloff, 2005). The higher abiotic pressure in the alpine setting, on the other hand, can be interpreted as constraining functional dispersion among species. Accordingly, only species sharing similar traits may survive in the harsh alpine environment (Machac et al., 2011; Reymond et al., 2013). Indeed, the more frequent occurrence of subordinate species at higher altitudes (*Formica lemani, Myrmica lobulicornis* and *Tetramorium alpestre*) indicates that abiotic filtering through harsh climatic conditions might be an important force to shape ant assemblages in the alpine environment.

The CWMs related to the feeding preferences of the ant community revealed a general reduction in the usage of all possible sources when moving downhill from the alpine grassland to the forest, except for the role of trophobiosis. Hence, despite the somewhat coarse quality of available data (relative ant abundance scores and qualitative literature records on feeding preferences: Seifert, 2017) a clear ecological pattern emerged. This increase in the role of trophobiotic associations in the forest corroborates the results of the baiting experiment and of the isotope analysis discussed below. The higher score of carnivore feeding on the tree line (not significantly higher than at the alpine sites) is an effect of the higher abundance of Myrmicinae, most species of which feed predominantly on other arthropods (Seifert, 2017). Also the higher usage of flower nectar by ants could be a sign that carbohydrates are limiting in the alpine setting and less rewarding sources are used more intensely. Since ants are found pollinating some alpine flowers (Claessens & Seifert, 2017) and were often seen visiting several different flowers (personal observations, unpublished) this result makes also sense. Interestingly, the patterns of trophobiosis as opposed to carnivorous feeding modes are clearly complementary to each other.

The foraging strata where the majority of the ant community searches for food are analogous to the feeding traits and show an increased soil surface foraging, as well as foraging in the herb layer above the tree line, and a complementary increase in the foraging on trees within the forest.

All analysed traits about ant morphology were clearly increasing in the forest (head size and colony size), and both these traits reflect the dominance hierarchy within ant communities. According to Savolainen & Vepsäläinen (1988) in the forest the mound-building wood ants clearly drive the composition of ant assemblages as the single most dominant representatives.

4.4. Resource usage and limitations

The feeding habits of ants have been already widely explored within several different habitats worldwide (e.g. Blüthgen & Fiedler, 2004; Kaspari et al., 2012; Peters et al., 2014; Orivel et al., 2018); but for the alpine settings the literature is surprisingly slim (but see Spotti et al., 2015; Guariento et al., 2018). Usage of resources was similar in all sampled sites, slopes and habitats with a clear general preference for sugar solutions (just sugar and also in a mixture together with glutamine; Fig. 16). The usage of the other offered recourses revealed amino acids and salt at a medium rank, followed by water and lastly by lipids. The relative usage of these resources changed slightly over the ecotone in the way that sugar was less intensely used in the forest (significant interaction; Fig. 17; Tab. 12). This pattern was mainly driven by *Formica* s. str. and less so by *Formica lemani.* The reduction in sugar usage was met with a relative increase in the usage of salt and also amino acids, although the usage of these two resources did not change significantly over the ecotone. Blüthgen & Fiedler (2004) and Peters et al. (2014) found a

tendency of tropical ants to reject glutamine as sole amino acid in nectar-mimic baits. This could have been an issue also in the present study, where this amino acid was offered pure. Nevertheless, the usage of glutamine was very similar to the usage of salt solution, probably indicating the general need for prey items. The usage of the baits with amino-acids and sugar mixture was nearly exactly the same as visitation just on pure sugar baits, suggesting that sugar was the main cause of ant attraction to this bait. However, the mixture baits were visited more evenly over the gradient than the sugar baits (especially by *Formica* s. str.) suggesting that the mixture itself made them more attractive than just sugar or amino-acids. For other species of the Formicinae subfamily it was found that exactly a mixture of sugars and amino acids together enhances the recruitment (Detrain et al., 2010).

Complementarity Theory (Kay, 2002, 2004) predicts that a resource is inversely used relative to the availability for an organism in its habitat. Hence, if a resource is limited, its usage will be proportionally higher. Under this framework, results of my baiting experiments indicate that sugar (i.e. energy related resources) were more limiting for alpine ants in general. The expectation that within the forest this limitation was relaxed was met, most probably because of the higher presence of aphids on trees (Tab. 16), which supplied significantly more sugary excretions. In the alpine setting, in contrast, this limitation seemed to be higher and sugar was more intensely visited. This higher usage of carbohydrates in alpine settings were also found by Spotti et al. (2015) and Guariento et al. (2018) and appears to be a consistent characteristic of the ant fauna of this habitat. Since it is known that ants with a more carnivorous lifestyle are more limited in sugar (Kaspari et al., 2012), alpine ants seem to have a generally more carnivorous feeding habit than their relatives at lower elevations.

Carnivorous arthropods should also be limited in lipids (Wilder et al., 2013). Indeed, Peters et al. (2014) found such a limitation with increasing altitude on an afromontane altitudinal gradient. In the present study, however, lipids were clearly avoided and were even visited less frequently than water. This rejection of lipids by ants in the European Alps was also found by Spotti et al. (2015) and Guariento et al. (2018) and again seems also to be a consistent characteristic of alpine ants. Other studies on further alpine settings worldwide could deliver a more comprehensive picture of lipid and sugar limitation in these harsh habitats.

An increased usage of amino acids and sodium should, on the other hand, indicate a more herbivorous feeding habit (Kaspari et al., 2008). The tendency of increased usage of sodium, but even more so of amino acids in the forest might be interpreted as a sign of a lower trophic position of ants in the forest.

Water was used slightly more often in the forest, but with a very low increase and the total number of visitations on this control resource was quite small (33 incidences in total). This was not surprising since in the European Alps, with their frequent rainfall events during summer, water will hardly ever be in short supply to the local ant fauna. Overall, the results of my baiting experiments can well be understood in the framework of *Complementarity Theory*.

Yet, alternative hypotheses might help to further understand the results and relate them to other studies, for example the *Economics Hypothesis* (Yoshida, 2006) and the *Metabolic Fuel Hypothesis* (Davidson, 1997; Kay et al., 2010). The *Economics Hypothesis* predicts that resource availability drives communities towards a concentration of related traits to exploit particularly important resources, and so the presence of species and the composition of communities change accordingly. In line with this idea, the higher frequency of trophobiosis partners would be responsible for the stronger expression of traits at the community level related to interacting with

homopterans (especially wood ants in forest; Timo et al., 2016). Further support for this hypothesis come from the observation of trait changes (especially feeding preferences and foraging strata; Fig. 15) described above. Hence, the expectation that changing resources availabilities (like the increase of carbohydrate supply in the forest) drives a different community composition (Fig. 13) with a different set of traits (CWMS) was met in the present study.

The *Metabolic Hypothesis* predicts that with a surplus of carbon available (viz. energy supply), it will be invested by ants to sustain higher activity and more aggressive colonies (Yanoviak & Kaspari, 2000; Gibb & Cunningham, 2009). Indeed, some studies found that with more C availability and its usage (and accordingly a more herbivorous feeding behaviour) the size of ant workers tended to increase (Davidson, 2005). In my study, the stronger prevalence of wood ants in the forest, which are bigger (both in colony and worker size) and more dominant than all other ant species found (Savolainen et al., 1988), well fits the concept of the *Metabolic Hypothesis*.

The wood ants, as the by far most dominant species in the studied systems, are expected to fulfil their colony needs irrespective of the occurrence of other ant species. Accordingly, the pattern of resource preference of this subgenus was quite clear and indeed changed in the expected manner from the alpine setting down to the forest (Fig. 19). In contrast, the subordinate alpine slave ant, *Formica lemani*, is known to switch its feeding behaviour in the presence of the wood ants to a more opportunistic one (Johansson & Gibb, 2016). The lack of a clear altitudinal change in the feeding habits of this species could be interpreted as a sign of the influence of the wood ants. *Formica lemani* visited more the mixture bait as the wood ants and amino acid were visited in the same proportion over the gradient. The alpine slaver ant is known to exploit fast new resources (Schiestl & Glaser, 2012) and was found on 77% of the visited baits, wood ants on the other hand just on 29%. The Myrmicinae group, that occurred on baits only very rarely (just on 15% of the visited baits), comprised a quite varied species assemblage (from the genera *Temnothorax*, *Leptothorax*, *Myrmica*, *Tetramorium* and *Manica*) and their feeding preferences will not be discussed further except for the fact that sugary baits were clearly preferred by this group over the whole gradient.

4.5. Stable isotope signatures

Results of stable isotope analysis largely corroborated the inferences drawn from the baiting experiment and the indirect trait analysis. Generally speaking, the expected trend from a more carnivore habit (i.e. higher trophic position) of the numerically and behaviourally dominant ants (*F. lemani* and *Formica* s. str.) in the alpine setting, to a lower (i.e. more herbivorous) position (of the very same species) in the forest was confirmed. The access to consistent trophobiotic partners that provide reliable sugar resources (directly linked to energy; Kaspari et al., 2012) seems to be a central aspect, enabling ants to access plant resources.

Further, the already known trend that, with increasing numbers of individuals (and for the tropics also of species) a reduction of the trophic position of the whole community happens (e.g. Davidson et al., 2003; Blüthgen & Feldhaar, 2010; Zhang et al., 2015), was here confirmed once again. The trend to a lower trophic position as a response to (or as reason for?) higher individual numbers is here found even intraspecifically within two different species occurring over the whole investigated gradient. This adaptation makes lots of sense since the usage of resources from a lower trophic position enables the maintenance of a greater number of individuals, because the required resources are present in a way higher amount as for strictly carnivorous animals.

Interestingly, isotope analysis did not reveal a differential reaction over the ecotone of the two analysed species (Fig. 21). As pointed out above, the feeding habits of slave ants were already found to partially change in the presence of wood ants in the sense that carbohydrates were preferred over proteins (Vepsalainen & Savolainen, 1990) and smaller pray items were collected (Savolainen, 1991) in coniferous forest of Scandinavia. This difference was not found with the isotope analysis, where the changes of the two species seem very similar to each other (Fig. 21). Further the generally lower isotope score of the alpine slave ant in comparison to the wood ant could have two meanings. First, of feeding more on plant resources and second, to feed on arthropods from a lower trophic position. The pray items of the smaller slaver ant are probably smaller, less defensive and from a lower trophic position as the arthropods on which wood ants feed on. For example woods ants probably ate ground active spiders that were found to negatively correlate with wood ants density. Savolainen (1991) found that slaver ants (*Formica fusca* in this case) switched to feeding on smaller arthropods in the presence of wood ants. A similar effect could have influenced the isotope fraction of the slave ants in the forest in the present study.

With regard to their isotope signatures, ants from the tree line locations scored more similar to those from alpine sites, although aphids occurred in greater numbers as well as interaction with them on the ecotone (more than in the alpine locations; Tab. 16). This result mirrors the outcome of the baiting experiment and the trait analysis, and seems to be a consistent characteristic of the ants' feeding habits at the tree line. The reason behind such a result can be searched in the relative contribution of trees to the feeding of the colonies on the ecotone. Les trees were present on the tree line and were visited more often (75% of the trees present were visited by ants) as the trees of the forest (52% of which had ant visitations). This higher visitation of trees on the tree line indicates that the feeding on the trees (that were also smaller as the trees within the forest; Tab. 1) was probably not enough to sustain the colony, so that foraging on the soil or on shrubs, as in the alpine sites, contributed significantly influencing the stable isotope signature. Also the smaller size of wood ant colonies on the tree line (as in the alpine settings) suggests a limited access to carbohydrates in comparison to the forest colonies. Further investigations are here necessary to shed light on the reasons driving this ecotone effect on nutrient limitations and feeding behaviour of ants.

It would be rewarding to test the aforementioned *Economics Hypothesis* (Yoshida, 2006) as well as the *Metabolic Fuel Hypothesis* (Davidson, 1997; Kay et al., 2010) intraspecifically within the two *Formica* species. This would allow to gain insight whether certain traits in feeding behaviour, body size, activity and colony aggressiveness might change according to the food restrictions in the different habitats. Since the feeding preferences on baits and isotope fractions did change, at least behaviourally there is a hint towards such an adaptation. Guariento et al. (2018) observed a reduced bait visitation at even higher altitudes within alpine grassland, indicating a reduced foraging activity of *F. lemani* that might result from reduced carbohydrate accessibility at higher altitudes within the alpine zone.

5. Conclusions

Overall the different hypotheses on the regulation of ant assemblages and their nutritional needs that were followed during this study were generally confirmed. The constraints on the ant community changed from the forest to the alpine setting. In the forest, competition pressure (exercised mainly by the mound-building wood ants) limited the presence of other ant species and

the ecological role of ants was in sum more shifted towards herbivore life styles. The main driver of the higher presence of wood ants (as well as for their lower trophic position) was a higher density of trophobiotic aphids, delivering substantial carbon (and so energy) supplies. In the alpine setting, on the other hand, abiotic filtering most probably driven by low temperatures, limited the presence of both ants and aphids. This led to change the overall feeding behaviour and community composition of ants to a more carnivore life style, but characterized by less competition. The tree line ecotone harboured an elevated species richness and functional diversity of ants. Yet, tree line ant scored surprisingly similar to those from alpine grassland, although due to the presence of trees and aphids at the tree line one would have expected these ant assemblages to functionally resemble more the situation in subalpine forest. This alpine similar scoring of the tree line is probably a result of a negative edge effect on the wood ants. Overall, the combination of different methods like community assessments, baiting experiments, stable isotope analyses and functional traits resulted largely concordant in the outcome and proved to be important to augment the functional understanding of alpine ant assemblages – which are still under-explored in most mountainous regions of the Earth.

6. Acknowledgements

7. References

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Online Resources
1. (www.martin361.com/geologiekarte_suedtirol. Last accessed: 05/04/2018).
2. (www.stelviopark.bz.it 05/04/2018)
3. (www.naturparks.provinz.bz.it/naturpark-texelgruppe.asp. Last accessed: 24/03/2018)
4. (www.provinz.bz.it/natur-raum/themen/managementplan-texelgruppe.asp. Last accessed: 05/04/2018)

Appendix

Table A1. GLMM Result of bait usages by Formica s. str. Only fixed factors are depicted (Bait type X Habitat). Slope, site nested in slope, transect nested in site and time of exposition end were modelled as random factors. The best model was selected using AICc (< 2), Akaike weights and R^2 marginal / conditional. Model scores: AIC 569; Degree of freedom of residuals 1095; R^2 marginal / conditional 0.92 / 0.94. *: significant at p<0.05; ****: significant at p<0.001.

Formica s. str.	Estimate	Std. Error	z value	p
(Intercept) Glutamine	-2.90	0.41	-7.07	< 0.001 ***
Mix	0.97	0.42	2.29	0.022 *
Oil	-17.09	2666.48	-0.01	0.995
Salt	0.70	0.44	1.59	0.112
Sucrose	0.78	0.43	1.81	0.070 .
Water	-0.74	0.64	-1.15	0.248
Habitat	-0.97	0.43	-2.23	0.025 *
Mix X Habitat	0.56	0.49	1.15	0.251
Oil X Habitat	-4.12	2673.67	-0.00	0.999
Salt X Habitat	0.33	0.51	0.66	0.512
Sucrose X Habitat	0.92	0.50	1.84	0.066 .
Water X Habitat	-0.37	0.72	-0.52	0.603

Table A2. GLMM Result of bait usages by *Fomica lemani*. Only fixed factors are depicted (Bait type X Habitat). Slope, site nested in slope, transect nested in site and time of exposition end were modelled as random factors. The best model was selected using AICc (< 2), Akaike weights and R^2 marginal / conditional. Model scores: AIC 867.1; Degree of freedom of residuals 1094; R^2 marginal / conditional 0.95 / 0.96

F. lemani	Estimate	Std. Error	z value	p
(Intercept) Glutamine	-1.67	0.33	-5.12	< 0.001 ***
Mix	1.51	0.30	5.06	< 0.001 ***
Oil	-17.69	181.03	-0.10	0.922
Salt	-0.22	0.33	-0.66	0.509
Sucrose	1.18	0.30	3.87	< 0.001 ***
Water	-1.38	0.41	-3.41	< 0.001 ***
Habitat	1.27	0.32	3.95	< 0.001 ***
Mix X Habitat	0.18	0.35	0.53	0.593
Oil X Habitat	14.49	181.02	0.08	0.936
Salt X Habitat	0.11	0.39	0.29	0.770
Sucrose X Habitat	0.56	0.36	1.55	0.120
Water X Habitat	-0.34	0.48	-0.72	0.468

Table A3. GLMM result of bait usages by Myrmicinae. Only fixed factors are depicted (Bait type X Habitat). Slope, site nested in slope and transect nested in site were modelled as random factors. The best model was selected using AICc (< 2), Akaike weights and R^2 marginal / conditional. Model scores: AIC 290; Degree of freedom of residuals 1095; R^2 marginal / conditional 0.025 / 0.059. *: significant at p<0.05.; ****: significant at p<0.001.

Myrmicinae	Estimate	Std. Error	z value	p
(Intercept) Glutamine	-3.2491	0.4140	-7.849	< 0.001 ***
Mix	0.9567	0.4178	2.290	0.022 *
Oil	-17.0696	104.6675	-0.163	0.870
Salt	0.6883	0.4312	1.596	0.110
Sucrose	0.7611	0.4231	1.799	0.072
Water	-0.7316	0.6139	-1.192	0.233
Habitat	-0.8897	0.4230	-2.103	0.035 *
Mix X Habitat	0.5288	0.4760	1.111	0.267
Oil X Habitat	0.8908	137.0419	0.006	0.995
Salt X Habitat	0.3099	0.4917	0.630	0.528
Sucrose X Habitat	0.8768	0.4843	1.810	0.070 .
Water X Habitat	-0.3598	0.6875	-0.523	0.601

Table B. Species occurrence and abundance of ground beetles found in the pitfall trap on each location (determined by Dominik Rable MSc; Nomenclature according to www.fauna-eu.org).

Ground Beetles Species	Z1	Z2	Z3	Z4	Z5	O1	O2	O3	O4	O5	U1	U2	U3	U4	U5	T1	T2	T3	T4	T5	M1	M2	M3	M4	M5
Abax exaratus Dejean, 1828	X																								
Amara lunicollis Schiodte, 1837													X												
Calathus melanocephalus Linne, 1758									X		X											X	X		
Calathus micropterus Duftschmid, 1812			X	X	X			X					X			X	X	X	X					X	
Carabus germarii Sturm, 1815		X														X	X								
Carabus problematicus Herbst, 1786									X																
Cymindis vaporariorum Linne, 1758							X												X		X				
Pterostichus burmeisteri Heer, 1838	X	X	X													X	X	X	X						
Pterostichus jurinei Panzer, 1803		X		X	X											X	X		X						
Pterostichus multipunctatus Dejean, 1828						X	X	X	X	X		X	X	X	X							X		X	X
Pterostichus unctulatus Duftschmid, 1812	X						X		X	X			X	X	X	X	X	X					X		
Notiophilus biguttatus Fabricius, 1779												X													

Table C. Species occurrences of plant lice and plants and their interaction with ants and the plant on which they were found (Nomenclature according to www.fauna-eu.org).

Aphid species	Ant species	Plant species	Z1	Z2	Z3	Z4	Z5	O1	O2	O3	O4	O5	U1	U2	U3	U4	U5	T1	T2	T3	T4	T5	M1	M2	M3	M4	M5
Adelges laricis Vallot, 1836	*F. lemani*	*Larix decidua, Picea abies*					X			X	X					X									X		
Cinara cembrae Seitner, 1936	*F. lemani, F. lugubris, F. aquilonia*	*Pinus cembra*							X	X	X		X	X	X	X											
Cinara cuneomaculata Del Guercio, 1909	*F. lugubris*	*Larix decidua*															X										
Cinara juniperi De Geer, 1773	*F. lemani, F. lugubris*	*Juniperus communis*						X	X				X	X	X								X				
Cinara laricis Hartig, 1839	*F. lemani, F. lugubris*	*Larix decidua*								X					X	X									X		
Cinara piceae Panzer, 1800	*F. lugubris*	*Picea abies*																X	X		X						
Cinara piceicola Cholodkovsky, 1896	*F. lugubris F. aquilonia*	*Picea abies*		X	X																	X					
Cinara pilicornis Hartig, 1841	*F. lemani, F. lugubris*	*Picea abies*																	X	X	X	X					
Coccidea	*Manica rubida F. lemani*	*Juniperus communis*											X	X	X												
Macrosiphum rosae Linnaeus, 1758	-	*Rosa pendulina*																	X								
Physokermes sp.	*F. lugubris not clear interaction*	*Picea abies*			X														X								
Trioza sp	-	*Hieraceum sp.*																						X			
Uroleucon sp. 1	-	*Solidago minima*					X																				X
Uroleucon sp. 2	-	*Campanula sheuzeri*											X														

Table C. Trait matrix with all traits used in the computation for the functional diversity. The coding differentiats between direct measuments (CH, Shape, Fresh weight, Colony size, N. European countries, latitude and altitudes) and proportions als between variations of the same trait (Nest poly/monogynie, Nest place, Feeding habits, Foraging strata and Dominance hierarchy).

Traits / Species	Formica lugubris	Formica aquilonia	Formica rufa	Formica exsecta	Formica lemani	Camponotus herculeanus	Tetramorium alpestre	Temnothorax tuberum	Leptothorax acervorum	Manica rubida	Myrmica ruginodis	Myrmica lobicornis	Myrmica lobulicornis	Myrmica sulcinodis
Head size (CH)	1735	1504	1812	1391	1268	2487	748	624	816	1548	1209	1062	1073	1260
Shape	2.561	2.561	2.561	2.561	1.94	1.714	1.154	1.54	1.48	1.519	1.419	1.419	1.419	1.419
Fresh weight	13.38	8.71	15.24	6.89	3.96	26.36	0.483	0.374	0.804	5.635	2.566	1.739	1.794	2.905
Nest Polygyne	1	1	0.5	1	0.5	0.5	1	1	1	1	1	0	NA	0.5
Nest Monogyne	1	0	1	0.5	1	1	0.5	1	1	0	1	1	NA	0.5
Colony Monodom	1	0	1	0.5	0	0.5	0.5	1	1	1	0	1	NA	1
Colony Polydom	1	1	0.5	1	1	0.5	1	0	1	0.5	1	0		0.5
Colony size (n. workers)	60000	150000	60000	25000	1500	3000	15000	150	180	500	800	280	600	460
Colony founding as social parasite	1	0.5	1	1	0	0	0	0	0	0	0	0	0	0
Nest in soil	0.77	0.77	0.77	0.95	0.74	0.09	0.85	0.25	0.03	0.93	0.48	0.75	0.5	0.71
Nest in rock	0.02	0.02	0.02	0	0.05	0.01	0.05	0.42	0.04	0.02	0	0	0.1	0.03
Nest in moss	0	0	0	0	0.01	0	0	0.15	0.04	0	0.07	0.03	0.05	0.05
Nest in turf	0.01	0.01	0.01	0.05	0.12	0	0.1	0.06	0	0.05	0.1	0.1	0.3	0.2
Nest in litter	0	0	0	0	0	0	0	0.1	0	0	0.07	0.08	0	0.01
Nest in micro-space	0	0	0	0	0	0	0	0.09	0.01	0	0	0	0	0
Nest in wood	0.2	0.2	0.2	0	0.08	0.9	0	0.02	0.88	0	0.28	0.04	0.05	0.01
Carnivorous feeding	0.3	0.3	0.3	0.4	0.56	0.23	0.34	0.8	0.87	0.84	0.53	0.72	0.72	0.55
Feeding on nectar	0.03	0.03	0.03	0.02	0.11	0.05	0.05	0.15	0.02	0.03	0.02	0.04	0.04	0.08
Feeding on trophobiosis	0.65	0.65	0.65	0.56	0.27	0.67	0.36	0.05	0.07	0.11	0.3	0.2	0.2	0.3
Feeding on plant part	0.02	0.02	0.02	0.02	0.06	0.05	0.25	0	0.04	0.02	0.15	0.04	0.04	0.07
Foraging in the soil	0.05	0.07	0.07	0.21	0.16	0.06	0.51	0.07	0.21	0	0.16	0.17	0.17	0.22
Foraging in litter and moss	0.05	0.03	0.03	0.03	0.05	0.01	0.03	0.13	0.41	0.05	0.18	0.39	0.35	0.2
Foraging on the soil surface	0.3	0.3	0.3	0.43	0.4	0.13	0.43	0.27	0.36	0.7	0.26	0.39	0.38	0.45
Foraging on herbs	0.1	0.1	0.1	0.18	0.3	0.04	0.03	0.53	0.01	0.1	0.18	0.05	0.1	0.13
Foraging on the trees	0.5	0.5	0.5	0.15	0.09	0.76	0	0	0.01	0.15	0.22	0	0	0
Dominance hierarchy	3	3	3	2	1	2	2	1	1	1	1	1	1	1
N. European countries occurring in	32	27	42	39	31	37	5	37	39	16	41	39	8	33
Lowest latitude	42.1	41.72	40	40.55	36	36.63	44.33	35.11	35.25	36.57	36.77	39.73	42.52	37.58
Highest Latitude	70.02	70.38	63.5	70.37	70.91	71	47.5	65.18	71.5	50.6	71	71	47.88	71
Latitude range	27.92	28.66	23.50	29.82	34.87	34.37	3.17	30.07	36.25	14.03	34.23	31.27	5.36	33.42
Highest occurring altitude	2400	2400	2240	2400	3000	2400	2335	2300	3100	2300	2000	2100	2700	2300
Lowest occurring altitude	550	800	350	300	800	300	900	350	350	350	350	350	1000	800
Altitude range	1850	1600	1890	2100	2200	2100	1435	1950	2750	1950	1650	1750	1700	1500

Table D. Species of plants recorded at the sampling sites (letter denotes the slope and number the site with 1 and 2 being the alpine setting, 3 the tree line and 4 and in the woods (revised by Julian Heider MSc; Nomenclature according to www.infoflora.ch).

Plant species	Z1	Z2	Z3	Z4	Z5	O1	O2	O3	O4	O5	U1	U2	U3	U4	U5	T1	T2	T3	T4	T5	M1	M2	M3	M4	M5
Achillea millefolium agg.			X																						
Achillea moscata L.s.l.						X	X																	X	
Antennaria dioica (L.) J. Gaertn	X	X	X			X	X	X		X	X	X	X		X						X	X	X	X	
Anthoxanthum odoratum L.s.l.									X		X										X	X			
Arctostaphylos uva-ursi (L.) Spreng.			X		X	X	X	X			X	X												X	
Arnica montana L.			X		X													X					X	X	
Avenella flexuosa (L.) Drejer	X		X	X			X	X						X									X	X	X
Botrychium lunaria (L.) Sw.						X	X																	X	
Calluna vulgaris (L.) Hull	X	X	X			X	X	X	X		X	X	X	X			X	X				X		X	
Campanula barbata L.	X	X	X	X		X	X	X	X	X		X	X	X							X	X	X	X	X
Campanula scheuchzeri Vill.	X	X	X	X	X						X	X	X	X	X									X	X
Carlina acaulis L.s.l.	X	X	X	X	X							X		X									X	X	X
Cerastium arvense L.s.l.						X	X		X							X	X								
Chaerophyllum villarsii W. D. J. Koch																X	X								
Clematis alpina (L.) Mill.																X									
Coeloglossum viride (L.) Hartm.	X	X				X			X							X	X								
Cystopteris fragilis (L.) Bernh.																								X	
Daphne striata Tratt.						X	X															X	X	X	X
Deschampsia cespitosa (L.) P. Beauv.										X											X				
Erigeron alpinus L.																								X	
Euphrasia minima Schleich.	X	X	X			X	X	X	X	X	X	X	X	X	X						X	X	X	X	X
Galium anisophyllon Vill.	X	X																							
Gentiana nivalis L.						X	X																		
Gentianella ramosa (Hegetschw.) Holub	X	X				X	X	X			X	X	X	X	X							X	X	X	
Gentiana verna L.																X	X		X					X	
Geranium sylvaticum L.																X									
Geum montanum L.	X	X							X			X	X									X	X	X	X
Gymnocarpium dryopteris (L.) Newman			X	X									X		X										
Helianthemum nummularium (L.) Mill.	X	X	X			X	X		X					X											
Hieracium glanduliferum agg.							X																		
Homogyne alpina (L.) Cass.							X	X					X	X				X	X					X	X
Huperzia selago (L.) Schrank & Mart.																		X	X						
Hypericum maculatum Crantz s.l.		X														X	X								
Hypericum perforatum L. s.l.			X	X																					
Juncus trifidus L.		X	X	X							X	X				X	X	X	X				X	X	
Juniperus communis subsp. *alpina* Celak.	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X
Knautia arvensis (L.) Coult.			X																						
Larix decidua Mill.	X		X	X		X	X	X	X	X	X	X	X	X							X	X		X	X
Leucanthemopsis alpina (L.) Heywood	X																							X	X
Ligusticum mutellina (L.) Crantz			X																						
Loiseleuria procumbens (L.) Desv.		X								X															
Lotus corniculatus agg.	X	X	X			X	X	X	X	X						X	X				X	X		X	X
Lupinus polyphyllus Lindl.							X															X	X	X	X
Luzula luzulina (Vill.) Dalla Torre &										X						X	X	X			X				
Luzula luzuloides (Lam.) Dandy &	X	X	X	X				X				X	X					X	X						
Luzula sylvatica agg.																									
Maianthemum bifolium (L.) F. W. Schmidt								X				X	X					X	X						
Melampyrum sylvaticum L.			X				X	X				X	X					X	X	X					
Myosotis alpestris F. W. Schmidt																X	X								
Nardus stricta L.	X	X	X	X		X	X	X			X	X	X	X				X	X			X	X	X	X
Pedicularis kerneri Dalla Torre									X																
Pedicularis tuberosa L.																X	X	X							
Phleum rhaeticum (Humphries) Rauschert														X		X	X	X					X		
Phyteuma hemisphaericum L.					X	X	X	X	X					X										X	X
Phyteuma spicatum L.														X											
Picea abies (L.) H. Karst.	X	X	X	X	X		X	X	X	X		X	X	X		X	X	X	X	X					X

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Pinus cembra L.						X	X	X	X	X	X	X	X	X	X							X	X			X
Polygonum viviparum L.						X	X	X	X																	
Polypodium vulgare L.			X																							
Polystichum lonchitis (L.) Roth					X																					
Populus nigra L.					X																					
Potentilla aurea L.																		X	X							
Potentilla erecta (L.) Raeusch.		X	X	X													X			X	X	X				
Pulsatilla alpina (L.) Delarbre s.l.													X					X	X							
Ranunculus acris L.								X																		
Ranunculus villarsii DC.		X	X															X	X							
Rhododendron ferrugineum L.	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X
Rosa pendulina L.																			X							
Rubus idaeus L.		X		X						X										X	X	X				
Rumex scutatus L.																		X								
Sagina saginoides (L.) H. Karst.					X																					
Sempervivum arachnoideum L.				X																						
Sempervivum montanum L.		X				X	X	X	X			X						X	X				X	X	X	
Sempervivum tectorum L.													X		X	X										
Senecio abrotanifolius L.						X	X		X														X	X	X	
Senecio incanus L.													X													
Silene acaulis (L.) Jacq.	X	X											X												X	
Silene vulgaris (Moench) Garcke									X									X	X							
Solidago virgurea subsp. *minuta* (L.)						X	X	X					X	X										X	X	X
Sorbus aucuparia L.					X															X	X					
Stellaria holostea L.		X	X																							
Thymus pulegioides L.	X	X	X		X	X	X		X		X	X	X		X	X	X	X								X
Trifolium alpinum L.						X		X	X														X		X	
Trifolium badium Schreb.																		X								
Trifolium montanum L.						X	X																			
Trifolium pratense L.	X	X																X	X	X						X
Trifolium repens L.		X	X																							
Trollius europaeus L.																		X	X							
Vaccinium gaultherioides Bigelow	X	X				X	X	X			X	X	X						X							
Vaccinium myrtillus L.	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vaccinium uliginosum L.								X	X				X	X									X	X		X
Vaccinium vitis-idea L.	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				X	X	X	X	X	X		X
Veronica camaedrys L.																		X	X							
Veronica officinalis L.																	X									
Viola biflora L.																		X								