

# Local and landscape environmental heterogeneity drive ant community structure in temperate semi-natural upland grasslands

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## Abstract

Environmental heterogeneity is an important driver of ecological communities. Here, we assessed the effects of local and landscape spatial environmental heterogeneity on ant community structure in temperate semi-natural upland grasslands of Central Germany. We surveyed 33 grassland sites representing a gradient in elevation and landscape composition. Local environmental heterogeneity was measured in terms of variability of temperature and moisture within and between grasslands sites. Grassland management type (pasture vs. meadows) was additionally included as a local environmental heterogeneity measure. The complexity of habitat types in the surroundings of grassland sites were used as a measure of landscape environmental heterogeneity. As descriptors of ant community structure, we considered species composition, community evenness, and functional response traits. We found that extensively grazed pastures and within-site heterogeneity in soil moisture at local scale, and a high diversity of land cover types at the landscape scale affected ant species composition by promoting nest densities. Ant community evenness was high in wetter grasslands with low within-site variability in soil moisture and surrounded by a less diverse landscape. Fourth-corner models revealed that ant community structure response to environmental heterogeneity was mediated mainly by worker size, colony size, and life history traits related with colony reproduction and foundation. We discuss how within-site local variability in soil moisture and low intensity grazing promote ant species densities, and highlight the role of habitat temperature and humidity affecting on community evenness. We hypothesize that a higher diversity of land cover types in a forest-dominated landscape buffers less favorable environmental conditions for ant species establishment and dispersal between grasslands. We conclude that spatial environmental heterogeneity at local and landscape scale plays an important role as deterministic force in filtering ant species and, along with neutral processes (e.g. stochastic colonization), in shaping ant community structure in temperate semi-natural upland grasslands.

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evenness, and functional response traits. We found that extensively grazed pastures and within-site heterogeneity in soil moisture at local scale, and a high diversity of land cover types at the landscape scale affected ant species composition by promoting nest densities. Ant community evenness was high in wetter grasslands with low within-site variability in soil moisture and surrounded by a less diverse landscape. Fourth-corner models revealed that ant community structure response to environmental heterogeneity was mediated mainly by worker size, colony size, and life history traits related with colony reproduction and foundation. We discuss how within-site local variability in soil moisture and low intensity grazing promote ant species densities, and highlight the role of habitat temperature and humidity affecting on community evenness. We hypothesize that a higher diversity of land cover types in a forest-dominated landscape buffers less favorable environmental conditions for ant species establishment and dispersal between grasslands. We conclude that spatial environmental heterogeneity at local and landscape scale plays an important role as deterministic force in filtering ant species and, along with neutral processes (e.g. stochastic colonization), in shaping ant community structure in temperate semi-natural upland grasslands.

**Key words:** Formicidae, environmental filtering, evenness, species composition, pastures, meadows, fourth-corner models, response traits.

## 1. Introduction

Identifying the mechanisms underlying community assembly remains a central challenge in ecology. Ecological theory assumes that the dynamics and composition of communities are driven by the combined effects of environmental filtering (abiotic conditions), biotic conditions (inter- and intra-species interactions), neutral processes (dispersal limitations) and historical contingencies (speciation) at multiple spatial scales (Cavender-Bares et al. 2009, Götzenberger et al. 2012, Ovaskainen et al. 2017). Classical niche differentiation assumes environmental filtering and biotic interactions (mainly competition) as major mechanisms structuring local communities, selecting species with specific environmental requirements that allow them to survive and persist at a given location, but culling species unable to tolerate such conditions (Kraft et al. 2015, Cadotte et al. 2017). However, the “filtering” effect of the abiotic environment is sensitive to the spatial scale and therefore intimately related to the spatial heterogeneity of the environment (Kraft et al. 2015). Defining the spatial extent (local, landscape, regional) is therefore key to properly address the role of the environment and its variability in the filtering process of ecological communities (Kraft et al. 2015, Cadotte et al. 2017).

Environmental heterogeneity is a ubiquitous driver of ecological processes in natural and semi-natural systems (Costanza et al. 2011, De Bello et al. 2013, Stein et al. 2014). In a broad sense, environmental heterogeneity refers to all aspects of spatial heterogeneity, complexity, diversity, structure, or variability in abiotic and biotic environmental conditions (Stein et al. 2014, Stein and Kreft 2015), and is regarded as a primary mechanism explaining diversity patterns and species coexistence (Tilman 1982, Melbourne et al. 2007, Costanza et al. 2011). Spatial environmental heterogeneity is thought to promote species diversity through three major mechanisms (Stein et al. 2014, Stein and Kreft 2015): i) an increase of gradients or variability in the environment (with regard to the amount of resources, habitat types or structural complexity) should increase available niche space and allow more species to coexist; ii) more heterogeneous habitats are more likely to provide refuge from adverse environmental conditions, promoting species persistence; and iii) the probability of speciation events resulting from isolation or adaptation to diverse environmental conditions should increase with environmental heterogeneity. There is substantial evidence demonstrating that heterogeneity in abiotic and biotic environmental conditions plays a significant role in structuring communities by either deterministic and/or stochastic processes at local and landscape scale (Götzenberger et al. 2012, Brown et al. 2013, Bar-Massada et al. 2014). Although there is widespread empirical evidence supporting a positive effect of environmental heterogeneity on species diversity (Stein and Kreft 2015) and functional diversity (Stark et al. 2017, Price et al. 2017), the extent and generality of this positive relationships have been questioned by several studies (e.g. Tamme et al. 2010, Gazol et al. 2013, Laanisto et al. 2013).

The role of environmental conditions and heterogeneity in structuring ant communities (taxonomically and functionally) has been frequently addressed by means of climatic and habitat factors (Sarty et al. 2006, Sanders et al. 2007, Bernadou et al. 2014), particularly at regional and global scales (Lassau et al. 2005,

Gibb and Parr 2010, Arnan et al. 2017). For example, environmental filtering has been suggested as the main ecological mechanism structuring European ant communities at continental and biogeographic scale (Arnan et al. 2017, Boet et al. 2020), while habitat complexity and abiotic variation along environmental gradients have been shown to shape taxonomic and functional diversity of ants in warm-temperate Mediterranean regions (Arnan et al. 2014, Blatrix et al. 2016). A recent study conducted in differently structured urban green spaces further indicated that structural complexity of the local vegetation can act as an environmental filter, driving ant communities in terms of species numbers and functional traits (Nooten et al. 2020). Comparatively few studies have been conducted in managed temperate grasslands by directly addressing the environment-community structure relationship at different spatial scales (Dauber et al. 2003, Dauber and Wolters 2005, Dahms et al. 2010) and even fewer have included a functional trait-based approach (van Noordwijk et al. 2012, Heuss et al. 2019, Scharnhorst et al. 2021).

Ants are an important and omnipresent component of biodiversity in grasslands, and constitute major aboveground generalist predators (Seifert 2018, Sanders and van Veen 2011, Wills and Landis 2018). They are considered ecosystem engineers, directly or indirectly controlling many ecosystem processes by altering physical, chemical, and biological soil properties at their nesting sites (Frouz and Jilková 2008, Sanders and van Veen 2011, Wills and Landis 2018). In European temperate grasslands, ant communities have mostly been described and analyzed with regard to land-use impact on species richness and abundance (e.g. Dahms et al. 2005, Pihlgreen et al. 2010, Pérez-Sánchez et al. 2018). Although there is solid evidence suggesting that land-use intensification (increased grazing, mowing and fertilization) decreases ant richness in temperate grasslands (Heuss et al. 2019), many studies suggest that local differences in micro-climate and soil conditions influence ant communities more strongly than direct management practices (Dahms et al. 2005, Seifert 2017, Pérez-Sánchez et al. 2018). In fact, a site-dependent response of ant communities to management is a strikingly common outcome in almost all research efforts so far, even in large-scale studies across wider geographic gradients (Seifert 2017, Pérez-Sánchez et al. 2018, Heuss et al. 2019). This site-dependent pattern has been explained by how management practices or their absence affect biotic (e.g. physical structure of vegetation) and abiotic (e.g. micro-climate heterogeneity) conditions for ants in a local context (Seifert 2017, Pérez-Sánchez et al. 2018), which can also be understood as how environmental heterogeneity drives ant community composition at within-site or local scale. Consequently, environmental variability, including heterogeneity induced by management, at local (within grasslands) and landscape (surroundings) scale may play a major role in shaping ant communities in European temperate grasslands.

Here we assessed the effects of local and landscape spatial environmental heterogeneity on ant community structure in temperate semi-natural upland grasslands of Central Germany. We used “environmental heterogeneity” as an umbrella term (*sensu* Stein and Kreft 2015) to describe the variability in temperature, soil moisture, and management type (pasture vs. meadow) within and between grassland sites (local environmental heterogeneity), as well as the complexity of habitat types in the surrounding landscape (landscape environmental heterogeneity). As descriptors of ant community structure, we focused on species composition in terms of nest density, community evenness, and selected ant species traits following a fourth-corner model approach (Brown et al. 2014). We addressed the following questions:

- i) Does ant species composition respond to local (within and between grassland sites) and landscape (grassland site surroundings) environmental heterogeneity?
- ii) Is ant community evenness (independently of species identity) positively affected by environmental heterogeneity at both spatial scales?
- iii) Which species traits mediate the response of ant community structure to environmental heterogeneity?

We expect ant community structure to be determined by environmental heterogeneity at both local and landscape scale, with a differential response on species composition but an increase in community evenness along with environmental heterogeneity. Similarly, we expect that the response of ant community to environmental heterogeneity can be explained by ecological mechanisms involving not only a set of species morphological traits but also species ecological and life history attributes (van Noordwijk et al. 2012, Gibb

et al. 2015, Retana et al. 2015).

## 2. Material and methods

### 2.1. Study area

The study area is situated in the Thuringian Forest National Park, in the vicinity of the city of Zella-Mehlis, Germany (Figure A1 in the Appendix). The area is characterized by a steep elevation gradient ranging from 450 m (Zella-Mehlis) to c. 900 m above sea level (highest mountain peak). Mean annual temperature is 5 °C and mean annual precipitation is 1100 mm (Deutscher Wetterdienst 2017). The landscape is dominated by spruce forest (65%), followed by built-up areas (15%), extensively managed grasslands (11%), and small fragments of arable fields (3%). Grasslands are predominantly located in the surrounding of the city or along mountain valleys on steep slopes. Some grassland sites are isolated from each other by forests and have been traditionally used for haymaking, while others are connected by rotational extensive grazing to allow moving livestock from one pasture to the next. We selected 33 grassland sites that represent a gradient in elevation and landscape composition in the region (Figure A1). All selected sites were managed either as extensive pastures or extensive meadows, with no history of management intensification (in terms of increased livestock density or mowing rate, mineral fertilizer or pesticide use) or land abandonment (in terms of woody plant encroachment) in the last decades.

### 2.2. Ant survey and nest density

Ant assessment was based on Seifert's (2017) sampling method for non-arboreal ant species in Central Europe. This procedure consists of direct localization of workers and nests within a spatially nested scheme covering three levels of search effort in a specific area: an intensive scrutiny (*S*-) search performed on soil and vegetation within a smaller area (*S*- sampling area); a quick (*Q*-) search on ground surface performed within a larger area (*Q*-sampling area); and a spot inspection (*SI*-) in the most promising habitats for nests in the surroundings of the *Q*-areas (Seifert 2017). The *S*- search aims to detect nests of small species with hidden nests and small territories, while the *Q*- search reflects realistic nest densities of larger species with lower nest densities but larger territories (Seifert 2017). The *SI* sampling allows discovering nests of rare species such as social parasites of *Lasius* or *Formica* genera (Seifert 2017). We employed fixed dimensions of 64 m<sup>2</sup> for *S*- sampling areas and 400 m<sup>2</sup> for *Q*- sampling areas, while *SI* areas covered c. 900 ± 82 m<sup>2</sup>. The combination of these three levels constituted a sample unit referred to hereafter as Seifert-plot. Time expenditure for ant searching in *S*- sampling areas varied from 30 to 60 minutes depending on the vegetation structure, and up to 180 minutes for the entire Seifert-plot. Recording of foraging workers and nests was performed sequentially from *S*- to *Q*- and *SI*- areas, and up to 10 workers per nest were collected after finishing each sampling area. Depending on the grassland size and accessibility, one to three Seifert-plots were established per grassland site. All Seifert-plots were searched between 08:00 and 18:00 hrs local time in August 2017. All specimens collected were fixed in ethanol 90% and determined to species level using identification keys in Seifert (2018).

Nest counts from each Seifert-plot component were combined into a final integrated species-specific density following Seifert's (2017) method, which represents the nest density of a species within 100 m<sup>2</sup>. This integrated density is determined by allocating a species into a given *recording group*, which is a generalization of how perceptible a nest is based on the ant species biology (Seifert 2017). *Recording groups* describe the probability of finding a nest in each sampling level (*S*-, *Q*-, and *SI*-; Seifert 2017, 2018). Thus, a final integrated density value per grassland site was calculated as the sum of nests found in *S*-, *Q*- and *SI*- search levels divided by *apseudo-area* of the recording group to which a particular species belongs (Seifert 2017). *Pseudo-areas* are a weighting parameter calculated for each recording group separately and provide a measure of the total intensity of investigation on a certain Seifert-plot (Seifert 2017). Detailed information regarding integrated density calculation and sampling completeness assessment is provided in Box A1 of the Appendix. Data from each Seifert-plot were pooled at grassland site level, and a species by site matrix was constructed for further community analysis using integrated species-specific density (hereafter referred simply as nest densities) as entries.

## 2.3. Environmental heterogeneity measures

We divided environmental data into local and landscape environmental heterogeneity measures. The former subset comprised variability measures related to abiotic and biotic environmental conditions within and between grassland sites, while the latter subset included biotic land cover heterogeneity measures in the surrounding landscape of each grassland site (Stein et al. 2014; methodological details are provided in Table 1).

At the local scale, we calculated either the coefficient of variation [(standard deviation / mean)\*100] or range (max-min) of elevation (ELEV), surface aspect (ASP), surface slope (SLO), solar radiation (SRD) and topographic wetness index (TWI) within-site using a grid-based digital elevation model (10 m spatial resolution) derived from LiDAR data (German Office for Surveying and Geoinformation; Table 1). We additionally treated local grassland management type (Mtyp: pasture or meadow) as an environmental heterogeneity measure since both grazing and mowing lead to different levels of structural heterogeneity in vegetation and soil within grassland sites (Tälle et al. 2016). In order to account for potential local environmental filters involved in the distribution and establishment of ant species (Seifert 2017), we further included mean air temperature (Tmean), maximum SRD values (SRDmax), and mean TWI values (TWImean) per grassland site. Since area and environmental heterogeneity are often closely related (Stein et al. 2014), we additionally included grassland site area (Area) as a local variable to account for any confounding effect between both parameters. These last four variables were added to the local environmental heterogeneity subset as *between-site heterogeneity measures* to highlight abiotic conditions variability (environmental means *sensu* Stark et al. 2017) among surveyed grassland sites (Table 1).

To characterize environmental heterogeneity at the landscape scale, we calculated the landscape composition using digital thematic maps from German Real Estate Cadastre Information system (ALKIS) at a fine spatial resolution (1:5000). We calculated the percentage of land covered by forests, grasslands, arable land and built-up areas (roads, urban, and industrial areas) within a geodesic buffer of 250 m (edge to edge) for each grassland site (Table 1). Such buffer size provides an adequate spatial scale for evaluating the effect of landscape composition on ants in agricultural landscapes (Dauber et al. 2003). We additionally estimated the Shannon index of diversity (SHID) based on land cover types as proxy of landscape heterogeneity (Table 1).

## 2.4. Ant traits

According to our research questions, we focused on species traits that may reflect ant responses to biotic or abiotic environmental conditions (Lavorel and Garnier 2002, Violle et al. 2007). We selected seven response traits representing ant morphology, ecology, and life history in Central European grasslands (Table 2; methodological details are provided in Table A1). Response trait selection was based on previous findings by van Noordwijk et al. (2012), Retana et al. (2015), Seifert (2017), and Heuss et al. (2019). Trait data were pooled into a species by trait matrix for further statistical analysis (Brown et al. 2014).

## 2.5. Statistical analysis

All statistical analyses were performed using R version 3.6.3 (R Development Core Team 2020). Prior to the analysis, local and landscape environmental heterogeneity measures and traits were checked for collinearity issues using the pairwise Pearson correlation coefficient  $r$  (Dormann et al. 2013). All variables showed  $|r| < 0.7$  and were therefore considered as predictors in further analyses (Figure A2).

### 2.5.1 Community data exploration and evenness calculation

To illustrate variation in ant species composition within and between grassland sites, we performed a hierarchical cluster analysis using Euclidean distance and Ward's minimum variance as agglomeration method (Murtagh and Legendre 2014). We used the average silhouette criterion for internal validation of the cluster analysis (Rousseeuw 1987, Kaoungku et al. 2018). The cluster analysis was calculated and validated using

vegdist and hclust functions of Vegan R package version 2.5-0 (Oksanen et al. 2019), and fviz\_silhouette and eclust functions of the factoextra package version 1.0.6 (Kassambara and Mundt 2019).

Community evenness per grassland site was assessed by means of the relative evenness proposed by Jost (2010), which represents the amount of evenness relative to the minimum and maximum possible for a given richness. We used the relative logarithmic evenness (RLE) based on the diversity of order  $q$  also known as “true diversities” or “Hill numbers” (hereafter  ${}^qD$ ; Jost 2006, Jost 2010). The order ( $q$ ) of a diversity ( $D$ ) indicates the sensitivity of the measurement to common and rare species (Jost 2006): for  $q=0$ , the resulting value of a diversity ( ${}^0D$ ) is indifferent to species frequencies, favoring rare species by giving the same weight to all species in the community (e.g. species richness); for  $q=1$ , all species are weighted with their exact frequencies, and neither rare nor common species are favored in the resulting diversity value ( ${}^1D$ ; e.g. exponential of Shannon entropy index); and for  $q=2$ , species are weighted with the most frequent species in the community which favors more abundant species ( ${}^2D$ ; e.g. inverse of Simpson index). We chose the RLE of orders 0 and 2 ( $RLE_{0,2} = \ln^2 D / \ln {}^0D$ ), as it represents the proportion of the most abundant species in a community (Jost 2010). Both  ${}^0D$  and  ${}^2D$  are measures of effective number of species that satisfy the replication principle and account for the uniqueness of each species composing a community (Jost 2006, Gotelli and Chao 2013).  $RLE_{0,2}$  values range between 1 (perfectly even community) and nearly  $1/{}^0D$  (community dominated by one species; Jost 2010). Calculations of  ${}^qD$  were based on species nest density (see section 2.2) using PAST software version 3.25 (Hammer et al. 2001).

### 2.5.2. Testing community structure – environmental heterogeneity relationship

To test whether local and landscape environmental heterogeneity measures affect ant community structure, we applied generalized linear models for multivariate data (Wang et al. 2012). This method fits individual GLMs for each species using a common set of explanatory variables, and implements resampling-based hypothesis testing to make community-level and taxon-specific inferences about which environmental factors are associated with such multivariate data (Wang et al. 2012, Warton et al. 2015). We fitted multivariate GLMs for both predictor subsets (Table 2) and species-site abundance (nest counts) as response variable, using a negative binomial distribution and a log-link function. An offset term equals to mean *pseudo-area* (log-transformed) per grassland site was used in every model in order to interpret results in terms of nest density rather than raw nest counts (Warton et al. 2015). We used a backward step-wise model selection based on Akaike’s Information Criteria (AIC) in order to find the most parsimonious model for statistical inference (Burnham and Anderson 2002). The model with the smallest AIC was selected, and possible interactions were considered (Burnham and Anderson 2002). Dunn–Smyth residuals plotted against fitted values were used to check model assumptions (Wang et al. 2012). We used an analysis of deviance based on likelihood ratio statistics (LR) to test the significance of each predictor variable on ant community (“sum-of-LR” statistic; Wang et al. 2012, Warton et al. 2015). To account for correlation in nest density across species we used parametric bootstrapping (Monte Carlo, 999 bootstrap resamples), a method with good performance for small samples ( $n < 32$ ; Warton et al. 2017). Multivariate GLMs and significance testing were implemented using the functions `manyglm` and `anova.manyglm` in R package `mvabund` version 4.0.1 (Wang et al. 2019).

To assess the effect of local and landscape environmental heterogeneity measures on ant community evenness, we implemented beta regression models (Ferrari and Cribari-Neto 2004). Beta regression is a well-suited approach for modeling data that are bounded to the standard unit interval (0, 1) such as rates and proportions, and whose observations do not reach the limits of the interval (Ferrari and Cribari-Neto 2004). We fitted beta regression models for both predictor variable subsets using  $RLE_{0,2}$  as a response variable with a logit link function. Model selection and statistical inference were conducted as described above. Model assumptions were visually inspected in diagnostic plots of residuals and normal QQ-plots (Zuur et al. 2010). Beta regression models were calculated using the `betareg` function in R package `Betareg` version 3.1-2 (Cribari-Neto and Zeileis 2010), while the `lrtest` function from the R package `lmerTest` was used for testing likelihood ratios on nested models (Zeileis and Hothorn 2002).

### 2.5.3. Fourth-corner models

To quantify species responses to local and landscape heterogeneity measures and to understand their relationship with critical response traits, we applied a predictive fourth-corner approach proposed by Brown et al. (2014). This approach fits a single model to predict abundances across several taxa as a function of environmental variables, taxa (species) traits and their interaction (Brown et al. 2014, Löbel et al. 2018). Fourth-corner models were fitted using count data (back-transformed densities) and negative binomial distribution with a LASSO penalty estimated via cross-validation. The LASSO penalty automatically sets to zero any term in the model that does not explain any variation in species response (Brown et al. 2014). We first fitted a single predictive model for all ant species at all grassland sites assuming different environmental responses for different species (not attempting to explain responses using traits). This can be understood as a multi-species distribution model (multivariate SDM; Brown et al. 2014, Wang et al. 2019). Second, we fitted a fourth corner model by adding the trait term to the model equation in order to evaluate how differences in the responses of species to local and landscape environmental heterogeneity measures were mediated by traits. In order to assess the interaction strength and importance of each predictor variable on species density and traits, we plotted the standardized coefficients from resulting models (Brown et al. 2014, Gibb et al. 2015). Multivariate SDMs and fourth corner models were fitted using the `traitglm` function from `mvabund` package version 4.0.1 (Wang et al. 2019).

#### 4. Results

A total of 16 species, six genera, and 465 nests were recorded in 32 grassland sites. At one grassland site (G20, Figure A1) neither ants nor nests were detected. The average number of detected species was  $4.15 \pm 0.72$  (mean  $\pm$  95% CI) per grassland site (min= 1, max= 9; Figure 1). Total nest density varied between 0.22 and 22.7 with an average of  $7.85 \pm 2.29$  nest/ 100 m<sup>2</sup> per grassland site (Figure 1). Species with the highest nest density were *Myrmica scabrinodis*, *Myrmica rubra*, *Lasius niger*, *Formica fusca*, and *Lasius flavus* (Figure 1).  $RLE_{0,2}$  values showed a gradient in ant community evenness across grassland sites from even ( $RLE_{0,2}$  [?] 0.65, 54% of sites) to uneven communities ( $0.45 < RLE_{0,2} < 0.65$ , 35% of sites), with few sites having dominated communities ( $RLE_{0,2}$  [?] 0.45, 11%; Figure 1).

Cluster analysis revealed a pattern of species composition within grassland sites related to management type, total nest density, and their most dense species or group of species (Figure 1). The first division ( $k = 2$ ) generated one group of communities with high nest density in sites predominately managed as pasture and another group of communities with low density located in sites either managed as pasture or meadow (Figure 1). In the first group, a second division ( $k = 3$ ) separated uneven communities with high *M. scabrinodis* density (cluster 1) from sites with intermediate densities of this species but relatively high densities of other species (clusters 3, 6; Figure 1). A third division ( $k = 4$ ) in the second group separated grassland sites with high nest densities of *L. flavus* (cluster 4) from sites with even communities of rather low nest density (cluster 2; Figure 1), with a further division ( $k = 5$ ) of this branch grouping sites where *M. rubra* was the most dense species (cluster 5). A fifth division ( $k = 6$ ) split grasslands sites with intermediate densities of *M. scabrinodis* (cluster 3) from sites with high densities of *L. niger* (cluster 6). Internal cluster validation showed a decrease of the level of goodness and misplaced grassland sites with the increase of  $k$  clusters generation (Table A2). We considered  $k = 6$  as the most appropriated number of clusters with an overall  $S_i = 0.38$  and non-misplaced data points. Divisions with  $k > 7$  led to  $S_i = 0$  within clusters suggesting that the algorithm does not succeed in finding any ‘natural’ clustering (Rousseeuw 1987).

Multivariate GLMs showed that the variation in community structure is explained by environmental heterogeneity at both local (LR= 191.9, P= 0.001) and landscape (LR=64.1, P= 0.004) scale. Ant species composition was significantly affected by TWIcv, Mtyp, Tmean, TWImean, SHID and %Forest predictors (P< 0.05; Table A3). Multivariate SDMs showed contrasting effects of within-site soil moisture heterogeneity (TWIcv) on *Lasius* and *Myrmica* species, while management as pasture (Mtyp<sub>pasture</sub>) had an overall positive effect on species density, particularly strong for *L. niger* (Figure 2a). Warmer grassland temperatures (Tmean) promoted nest densities of *L. flavus*, *L. niger*, and *M. rubra*; while wetter grassland conditions (TWImean) had a negative effect on *L. flavus* nest density (Figure 2a). Diversity of land cover types (SHID) had a strong positive effect on nest densities of *Lasius* species, while surrounding forest cover (%Forest)

had contrasting effects on *L. flavus* and *F. fusca* densities (Figure 2b). Total nest density per grassland site increased with within-site soil moisture variability (TWIcv), management as pasture (Mtyp<sub>pasture</sub>) and diversity of land cover types (SHID) but a decrease with overall the increase of between-site soil moisture (TWImean; Figure A3).

Beta regression models showed that community evenness was affected by local (logLR= 18.8, P= 0.001) and landscape (logLR= 11.29, P= 0.04) environmental heterogeneity measures. Likelihood ratio tests showed that ASPrg, TWIcv, TWImean, SHID and %Forest had significant effects on community evenness (P < 0.05; Table A4). At the local scale, RLE<sub>0,2</sub> increased with within-site variability in surface aspect range (ASPrg) and between-site soil moisture (TWImean), but decreased with higher within-site soil moisture variability (TWIcv; Figure 3a). At the landscape scale, RLE<sub>0,2</sub> decreased with the increase of land cover diversity (SHID) and forest cover (%Forest; Figure 3b).

Fourth-corner models revealed a range of significant interactions between response traits and environmental predictors (Figure 4). The strongest community response to environmental heterogeneity measures was driven by worker size, colony size, behavioral dominance, and life history strategy traits (Figure 4). At the local scale, behavioral dominance was positively correlated with rugged slopes, while a generalist life history strategy was negatively correlated with soil temperature variability (Figure 4a). Pastures managed by low intensity grazing promoted species with aggressive behavior and species with a generalist strategy for colony founding (Figure 4a). A wide range of traits were correlated with soil moisture measures but to a lesser extent (Figure 4a). Species with small workers and species limited by food during colony foundation decreased in warmer grassland sites (Figure 4a). Species with large colonies and species with time-constrained and temperature limited nest foundation were favored by high temperatures (Figure 4a). At the landscape level, the strongest environment-trait interactions showed that higher landscape diversity had a negative effect on species with small workers, and higher forest cover in the surroundings hampered species with colony foundation dependent on temperature (Figure 4b).

## 5. Discussion

We assessed the effects of local and landscape environmental heterogeneity on ant community structure in semi-natural temperate grasslands. We found that extensively grazed pastures and within-site heterogeneity in soil moisture and topographic exposition at the local scale, as well as diversity of land cover types and forest cover at the landscape scale affected ant community composition and evenness. Our findings additionally confirmed the importance of local mean air temperature and mean soil moisture as environmental filters of ant species establishment in temperate grasslands (Dauber and Wolters 2005, Seifert 2017, Heuss et al. 2019). Furthermore, our trait-based approach showed that the response of overall ant community structure to local and landscape environmental heterogeneity was mediated by a set of species traits including morphological, ecological and life history features.

### 5.1. Effects of local and landscape heterogeneity on ant species composition

Within-site variability of soil moisture affected ant species composition by promoting nest densities of some species and therefore total nest density per grassland site, while an increase of mean soil moisture between grassland sites explained the decrease of nest densities in our studied system. Although both results on soil moisture may seem contradictory, they should be interpreted as the effect of soil moisture as *i*) an environmental heterogeneity measure reflecting variability within grasslands (TWIcv) and *ii*) an environmental means measure showing variability between grasslands sites (TWImean; *sensu* Stark et al. 2017). High levels of soil moisture have been reported to adversely affect ant species richness and density in Central European grasslands, with most ant species having a narrow range of tolerance to water content in soils (Dahms et al. 2005, Dauber et al. 2005, Seifert 2017, Heuss et al. 2019). In this sense, it seems likely that a higher environmental heterogeneity in terms of soil moisture (high TWIcv) exerts a positive effect on ant communities within grassland sites by providing more niche-spaces to be exploited by a wider group of species with different tolerances to wetness. On the other hand, the significant negative effect of mean soil moisture



on ants likely reflects the gradient in humidity between grassland sites in the study area, where less humid grasslands (lower  $TWImean$ ) accommodated more diverse ant communities than more humid grassland sites (higher  $TWImean$ ). This is in line with a more regional-scale pattern found across Central Europe, where ant diversity decreases from warm-dry to humid-cool grasslands (Dekoninck 2007, Seifert 2017). In contrast to soil moisture, the effect of mean air temperature was more evident at species than at entire community level (total nest density per site) where nest densities of the reportedly thermophilic species *L. niger*, *L. flavus*, *M. rubra* and *Formica cunicularia* (cf. Figure A4) increased along with  $Tmean$  (Seifert 2017, 2018).

In addition to the role of soil moisture, our findings showed that extensively managed pastures promote nest density of most of species compared to extensive meadows, which supports previous results highlighting the benefits of extensive grazing on biodiversity compared to annual mowing (Tälle et al. 2016). Although both extensive management types are expected to be beneficial for biodiversity in semi-natural grasslands, the mechanisms behind their effect on grassland vegetation and fauna may differ (Lepš 2014, Tälle et al. 2016). While low intensity (gradual but continuous) grazing by cattle creates a small-scale mosaic of disturbances in soil and spatial structure of vegetation (vertical and horizontal), annual mowing homogenizes the vegetation structure through consistent and uniform biomass removal in a short period of time (Olff and Ritchie 1998, Adler et al. 2001, Lepš 2014, Tälle et al. 2016). Thus, grazing disturbance not only enables openness throughout the sward but also creates a variety of different micro-sites and micro-climates suitable for a wider group of ground-dwelling insects, including ants (Cole et al. 2010, Hoffmann 2010, Jerrentrup et al. 2014). The differential effect of management types on grassland vegetation structure has been shown to affect ants indirectly by changing micro-habitat and soil moisture conditions (Dahms et al. 2005, Dauber et al. 2005, Pérez-Sánchez et al. 2018, Heuss et al. 2019), which correspond to our data showing higher within-site soil moisture heterogeneity in pastures ( $TWInc$ : mean = 14.7, SD = 5.9) than in meadows ( $TWInc$ : mean = 8.9, SD = 4.5). Thus, both environmental heterogeneity measures, management type and soil moisture variability, may address the same synergistic effect of providing favorable micro-habitat and soil conditions for ant communities.

At the landscape scale, a high diversity of land cover types had a general positive effect on species composition, while the influence of the surrounding forest varied among species. The effect of land cover heterogeneity on ant communities can be interpreted in two different ways. First, a higher variability in land cover types in the surroundings of the grassland sites may provide source habitats for new colonizing species, thereby enriching the local species pool (Benton et al. 2003, Öckinger et al. 2012). Second, a higher between-habitat heterogeneity in the surroundings increases the range of abiotic conditions, particularly along the edges, thus causing ecotonal effects that may favor thermophilic species near open habitat edges (i.e. arable, built-up land) or moist-tolerant species near shaded edges (i.e. forest) within grassland communities (Dauber and Wolters 2004). In our study region, the higher levels of landscape diversity are located at intermediate elevations where the land cover matrix transitions from a built up- to forest-dominated landscape and the proportions of neighboring arable lands and grasslands reach their peaks (Figure A1). Supporting the ecotonal effect, the observed species composition in many of these grasslands was characterized by species with different habitat preferences: *F. fusca* (woodland edges), *L. acervorum* (light forest), *F. cunicularia* (open verges), *L. niger* (urban and disturbed open habitats; Seifert 2018). The species-specific effects of surrounding forests detected in our study further support this statement, as *F. fusca* density was positively affected by %Forest, while densities of *L. flavus* and *F. cunicularia* were negatively affected (Figure A4).

## 5.2. Effects of local and landscape heterogeneity on ant community evenness

The effect of environmental heterogeneity on diversity has been explained by classical niche theory, where habitat heterogeneity is expected to increase species co-existence and therefore evenness (Stein and Kreft 2015). However, it has been argued that the high diversity of species in seemingly homogeneous habitats cannot be explained exclusively by niche processes, but also by neutral or stochastic processes related to demography and dispersal events (Hubbell 2001, Andersen 2008, Brown et al. 2013). In this regard, evenness has been used as neutrality metric, meaning that habitats with high levels of community evenness are subject to more neutral than deterministic processes (Schowalter 2011, Bar-Massada et al. 2014). In our study system, an

important proportion of grassland sites accommodate even communities, which suggests that neutral processes may also contribute to structuring ant communities in addition to environmental heterogeneity. The fact that ant response traits describing colony reproduction and dispersal constraints in semi-natural grasslands (van Noordwijk et al. 2012) were important in our fourth-corner analysis suggest that neutral processes, such as stochastic species dispersal, may contribute to the local assembly of ant communities. Nevertheless, these findings, although remarkably interesting, need to be considered with caution since neutrality levels in species-poor communities are difficult to interpret as the lower bound of evenness decreases with richness ( $1/D$ ), and in our studied system approximately 25% of the grassland sites showed communities with less than three ant species (Jost 2010, Bar-Massada et al. 2014).

In contrast to our expectations, the effect of environmental heterogeneity on ant community evenness was variable at local scale and negative at landscape scale. The effects of TWIcv, TWImean, and SHID on  $RLE_{0.2}$  were exactly opposite to those observed for species composition and density, hence their interpretation is intimately related to our previous discussion. An increase in within-site soil moisture variability and diversity of surrounding land cover types certainly supports total nest density, but also benefits numerical dominant species (i.e. *M. scabrinodis* or *L. niger*) which may lead to uneven or dominated ant communities in some grassland sites. Similarly, high levels of soil moisture may promote community evenness by limiting nest densities of numerical dominant ants in grasslands, which leads to a more even distribution of species densities within the community structure (c.f. sites in cluster 2, Figure 1). Model results additionally showed a positive effect of within-site surface aspect range (ASPrng) on community evenness. Our study system contains grasslands mostly facing from east to west through south azimuths. In grasslands facing east, the soil surface receives radiation earlier in the day when air temperature and evapotranspiration are lower leading to moister habitats (Ashcroft et al. 2008); while on west-facing sites, the soil surface reaches maximum temperatures during the afternoon when the direct radiation is at its maximum creating warmer and drier habitats (Bennie et al. 2008). A higher range of surface exposure within grasslands sites creates highly heterogeneous habitats for ant species, thereby increasing species co-existence and promoting ant diversity and community evenness.

### 5.3. Community structure response to environmental heterogeneity through species traits

The community structure response to environmental conditions is the result of different sets of species (response) traits, therefore quantifying how traits and environment interact provides a mechanistic understanding of community assembly (Lavorel and Garnier 2002, Zirbel et al. 2017). Our results show that the response of ant community structure to local heterogeneity on topography, soil temperature, and management was strongly related to behavioral dominance and a generalist life history strategy. Behavioral dominance may be an advantageous trait for food acquisition and territory defense in open habitats characterized by patchy vegetation such as pastures ( $M_{typ_{pasture}}$ ) with rugged slopes ( $SLO_{cv}$ ) where the probability of intra- and inter-specific competitive encounters is high (Savolainen and Vepsäläinen 1988, Savolainen et al. 1989, Pérez-Sánchez et al. 2018; but see Stuble et al. 2019). Similarly, species well adapted to deal with low food availability and variable soil temperatures during nest foundation (life history strategy G, according to van Noordwijk et al. 2012, cf. Table 2), may be favored in dynamic and heterogeneous habitats such as extensively managed pastures (Verberk et al. 2010). Surprisingly, we found a negative relationship between strategy G and within-site solar radiation range ( $SR_{Drg}$ ), expected to be positive according to the environmental heterogeneity hypothesis. We attribute this result to the overpowering negative effect of within-site soil temperature variability ( $SDR_{rg}$ ) on *L. flavus* densities, rather than the cumulative effects on all species with life history strategy G (i.e. *L. niger* and *F. fusca*; van Noordwijk et al. 2012).

Community response to local mean air temperature and soil moisture was strongly related to worker and colony size traits and, to a lesser extent, to life history strategies F and T (cf. Table 2). Higher mean temperatures negatively affected worker size, but positively affected colony size. The relationship between body size and temperature has been traditionally related to the heat conservation hypothesis, i.e. a larger body size has adaptive value in cold temperatures due to lower surface-to-volume ratios (Cushman et al. 1993, Blackburn et al. 1999). This hypothesis has been questioned for ants, as a larger body size in ectotherms also reduce the rate of heat gain, which is as important as decreasing heat loss (Cushman et al. 1993). However,

it may be suitable at the colony level as long as *i*) worker body size is positively correlated to colony size, and *ii*) colony size is correlated to thermoregulatory capabilities (Cushman et al. 1993, Kaspari and Vargo 1995). In our case, some *Formica* species meet such conditions and were certainly associated with cooler temperatures in upland sites or grasslands surrounded by forest (i.e. *F. fusca*, *F. pratensis*). Nevertheless, it is more likely that this particular result is influenced by species with small workers but large colonies such as *L. flavus* and *L. niger*, which showed high nest densities in grassland sites with high temperature records. On the other hand, the interaction of life history strategies with temperature suggests that grassland sites with high mean air temperatures have a low nutrient content for ants (strategy F), but may shelter species whose nest foundation is highly dependent on soil temperature (strategy T; van Noordwijk et al. 2012).

The strongest interactions between traits and environment were detected at landscape scale, with a functional response to land cover types (SHID) similar to local mean air temperature (Tmean). Although both predictors, Tmean and SHID, did not exceed the collinearity threshold ( $|r| < 0.7$ ; Dormann et al. 2013), their effect on ant communities seems to be related. In a forest-dominated landscape, a high number and even distribution of open land cover types (i.e. grasslands, arable lands and urban areas) in the surroundings likely increases air and soil temperature of grasslands; while a decrease in grassland temperatures can be expected when surrounding landscape is dominated by forest due to shading (Figure A2; Krämer et al. 2012, Öckinger et al. 2012, Liivamägi et al. 2014). The strong and negative relationship between life history strategy T and %Forest supports this statement, as species depending on high soil temperature for nest foundation showed low densities or were absent in grasslands surrounded completely or almost entirely by forest. In this sense, a high proportion of surrounding forests not only contributes to grassland isolation by imposing a physical barrier for species colonization, but also limits abiotic conditions for the establishment of grassland specialist and thermophilic species (Krämer et al. 2012, Öckinger et al. 2012).

## 6. Conclusions

Investigating the impacts of environmental heterogeneity on species diversity and functional traits at multiple spatial scales is crucial to better understand community patterns in ecology, and is especially relevant for biodiversity conservation (Costanza et al. 2011, Ovaskainen et al. 2017). This study shows that local environmental heterogeneity within and between grasslands, and the surrounding landscape play a relevant deterministic role in structuring ant communities in temperate semi-natural upland grasslands. Heterogeneity in habitat structure caused by grassland management (i.e. low intensity grazing) and within-site heterogeneity in soil moisture were the most important local environmental measures shaping ant species composition and density. At the landscape scale, a high land cover heterogeneity increased overall species density within grasslands, although the effect of each surrounding habitat type depended on species-specific abiotic requirements. The variation of community evenness against environmental heterogeneity showed an opposite pattern compared to community composition due to the inherent relationship between richness and evenness (Jost 2010). However, this approach revealed that a higher range of surface aspect (exposure of the slope) within grassland sites exerts a positive effect on community structure by promoting species co-existence. Overall, the proportion of even communities was high (54% of the surveyed sites) compared to uneven (35%) or dominated communities (11%), suggesting additional (neutral) processes operating on ant community assembly (Bar-Massada et al. 2014). The response of ant community structure to environmental heterogeneity at both spatial scales was mediated by morphological (worker size), ecological (dominant behavior) and life history (colony size and strategies related with colony reproduction- dispersal-foundation) traits. The interaction of the aforementioned response traits and the environment framed in a fourth-corner scheme proved to be a valuable approach for understanding how community structure is driven by environmental heterogeneity. We conclude that ant communities in semi-natural grasslands are not only driven by environmental heterogeneity but also by complementary neutral processes related to stochastic habitat colonization, as dispersal events between isolated grasslands are limited in a forest-dominated landscape. Both environmental heterogeneity and stochastic explanations are not exclusive and may represent opposite ends of the same continuous gradient denoting the relative contributions of both niche and neutral processes to the assembly of ant communities in temperate semi-natural grasslands (*sensu* Gravel et al. 2006).

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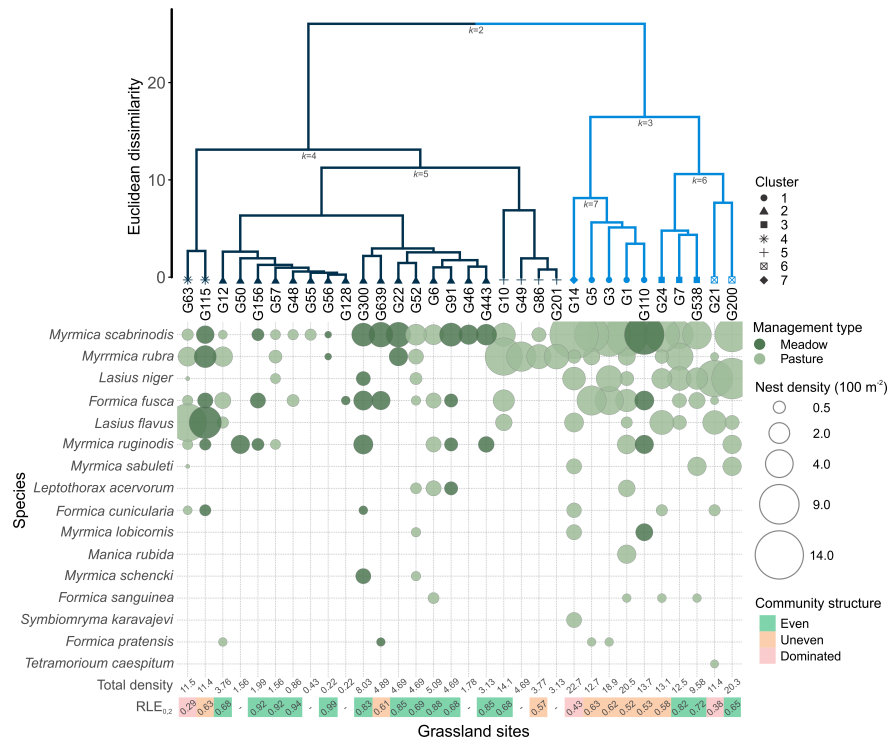
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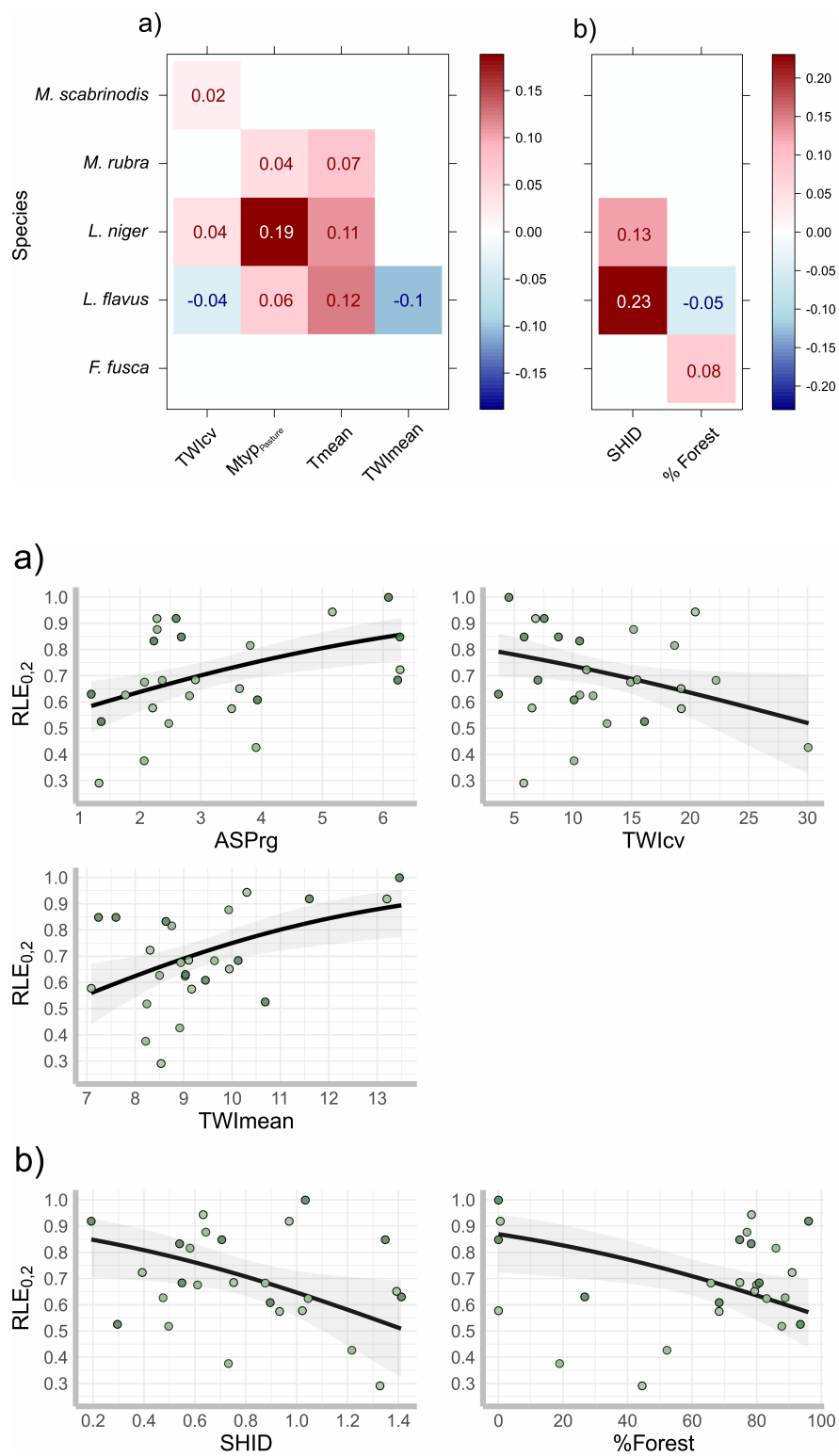
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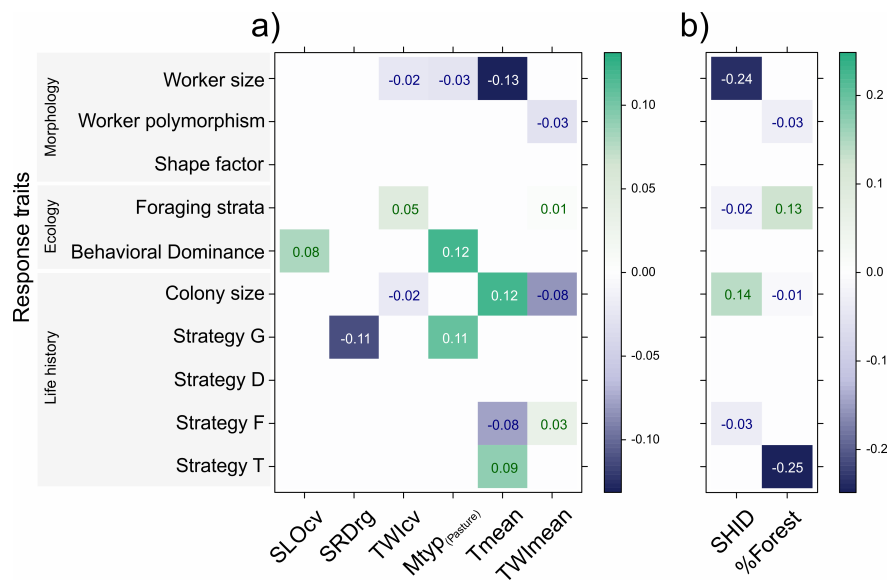
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