Evolutionary, ecological, and anthropogenic drivers of phenotypic diversity in ants

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Abstract

Evolutionary, ecological, and anthropogenic drivers of phenotypic diversity in ants

Javier Eduardo Ibarra Isassi, Ph.D.
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The drivers of phenotypic diversity have puzzled humanity for centuries. Functional trait approaches have helped advance the mechanistic understanding of the diversity of life forms. Previous work has shown that evolutionary history and environmental adaptation contribute to the observed diversity of phenotypes. However, most of our understanding comes from plants and studies that often neglect the influence of intraspecific variability. My thesis aims to investigate the drivers of phenotypic diversity across organizational levels using ants as study organisms. In Chapter 2, I examined the influence of evolutionary and environmental heterogeneity on the phenotypic diversity of ant lineages. I found a negative relationship between the diversity of climates occupied by ant genera and their phenotypic integration. This indicates that phenotypic integration may limit ant phenotypic diversification into new climatic zones. For Chapter 3, I examined geographic variation in community-wide patterns of phenotypic diversity, at different organizational levels (i.e., worker, colony, and species), along a 9° latitude gradient in Quebec, Canada. The results suggest that stressful environmental conditions typical of northern ecosystems exert a strong selection pressure on ant morphology causing shifts in optimal trait values of antennae length and eye size. Specifically, I found that shorter antennae and larger eyes possibly represent adaptations to cold, dry, and open habitats. In Chapter 4, I evaluated the impact of coffee plantation management practices on community-wide patterns of ant phenotypic diversity and composition. I found that intensified monocultures harbored phenotypically distinct ant communities. Moreover, community-wide phenotypic composition was more homogeneous in intensified plantations than in nearby forest patches or shade-grown plantations. This indicates that shade-grown strategies in coffee plantations buffer the impoverishment of ant phenotypic diversity following forest conversion, which could help preserve ecosystem services provided by ants. Overall, my thesis suggests that ant phenotypic diversity patterns are limited by phenotypic integration, vary among organizational levels (worker, colony, and species), and are influenced by anthropogenic disturbance across facets (taxonomic, phylogenetic, and functional). These findings have important implications for understanding how phenotypically complex organisms

respond to climate change and provide guidance for conservation strategies targeting vulnerable lineages.

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Contribution of Authors

As primary author, I contributed to the conception, planning, data collection/extraction, data analyses and writing for all thesis chapters. Similarly, Dr. Jean-Philippe Lessard and Dr. Tanya Handa contributed to the conception, planning, interpretation of results and reviewing of all chapters.

For chapter 4, co-authorship contribution is as follows: J.I.-I., conceived the ideas and statistical analyses; analysed data and led manuscript writing. I.T.H., J.-P.L. conceived the ideas and statistical analyses; I.A., S.E.-R., A.A.-C. conceived the methodology, collected data, and identified specimens; all authors contributed critically to the drafts and gave final approval for final version and publication of the manuscript.

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List of Abbreviations

ANOVA	Analysis of Variance
AUG	August
BERRY	Berry
CV	Coefficient of Variation
CWM	Community Weighted Mean
CWV	Community Weighted Variance
Dcentroid	Distance to centroid
DOMAI	Le Domaine
DORVA	Dorval-Lodge
DPINS	Chemin des Pins
DUPAR	Lac Duparquet
EASTM	Eastmain river
EL or MEL	Maximum Eye Length
EW or MEW	Maximum Eye Width
FAO	Food and Agriculture Organization
FL	Femur Length
GEE	Google Earth Engine
GIS	Geographic Information System
GLMM	Generalized Linear Mixed Model
GRS	Densitometer manufacturer
HAVEL	Havelock
HL	Head Length
HW	Head Width
IDEAM	Instituto de Hidrología Meteorología y Estudios Ambientales
JUL	July
KM	Kilometre
KM511	Baie Saint-James Road (KM 511)
LACDE	Lac Desaulniers
LASAR	La Sarre
MCC	Maximum Credibility Clade
MIRAB	Lac Mirabelli
ML	Mandible Length
MSBA	Mont Saint-Bruno (A)

MSBB	Mont Saint-Bruno (B)
MSHA	Mont Saint-Hilaire (A)
MSHB	Mont Saint-Hilaire (B)
NDVI	Normalized Difference Vegetation Index
NMDS	Non-Multidimensional Scaling
OBS	Observed
OUESC	Lac Ouescapis
PC	Principal Component
PCA	Principal Component Analysis
PCoA	Principal Coordinate Analyses
PERMANOVA	Permutational Multivariate Analysis of Variance
PERMDISP	Permutational Analysis of Multivariate Dispersion
PGLS	Phylogenetic Generalized Least Squares
PW	Pronotum Width
RADIS	Radisson
REDPI	Chemin du Pin Rouge
RUPER	Riviere Rupert
SD	Standard Deviation
SES	Standardized Effect Size
SESPI	Standardized Effect Size of Phenotypic Integration
SESVol	Standardized Effect Size of Volume
SL	Scape Length
SVM	Support Vector Machine
TIC/IR	Ratio of community-wide variance in regional pool
TIP/IC	Ratio of within-population variance to total within-community variance
TIP/IR	Ratio of community-wide variance to total variance of regional pool
TL	Tibia Length
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
VALDA	Val-David
WL	Weber's Length

Chapter 1 General Introduction

Biologists have long been using traits to study the relationship between organisms and the environment. Early studies aimed to categorize organisms based on how they respond to environmental variables or the strategies they use to reproduce, survive, and compete (Raunkiaer 1934, MacArthur and Wilson 1967, Grime 1974, Calow 1987). Later, studies shifted their focus towards predicting species distribution changes and ecosystem processes using traits (Diaz and Cabido 1997, Lavorel et al. 1997, Chapin et al. 2000). The term 'trait' refers to any morphological, behavioral, physiological, or phenological attribute that is measurable at the individual level (Violle et al. 2007). Traits related to the performance (i.e., growth, survival, and reproduction) of an individual and/or an ecological process are referred to as 'functional traits' (Violle et al. 2007, Lavorel 2013). Given these concepts, traits have been used to help explain the effects of climate change (e.g., Berg et al. 2010, Maire et al. 2015, Šímová et al. 2018), environmental gradients and stressors (e.g., Arnan et al. 2012, Dias et al. 2013, Vesk 2013) on species distribution, range shifts and community dynamics (e.g., Diaz et al. 2004, Cornwell et al. 2008, Cornwell and Ackerly 2009, Pollock et al. 2012, Valladares et al. 2014, Wieczynski et al. 2019). Indeed, traits can help us unveil the mechanisms underpinning the relationship between organisms and ecosystem processes across scales due to its ability to be generalized beyond taxonomic approaches (Lavorel and Garnier 2002, Violle et al. 2007, 2014, Messier et al. 2010, 2017, Carmona et al. 2016). Although plant ecologists have primarily led the validation of the utility of traits to detect the processes behind biodiversity patterns (e.g., Wright et al. 2004, Díaz et al. 2016, Messier et al. 2017, Bruelheide et al. 2018), their success has encouraged an increased focus on animal trait-based approaches to do the same (Moretti et al. 2017, Brousseau et al. 2018, Carmona et al. 2021). However, we still need to improve our understanding of the influence of processes such as selection and environmental adaptation that may operate across lineages and organizational levels, and how anthropogenic change is modifying these processes and, in turn, affecting observed diversity patterns.

The observed diversity of traits (i.e., phenotypic diversity) is the result of a combination of factors, including evolutionary conservatism, speciation in response to environmental pressures, and genetic, developmental, and physical constraints. Evolutionary conservatism implies that closely related lineages share phenotypic similarities due to the retention of ancestral traits, whereas ecological speciation promotes phenotypic innovation (Losos 2008, Ackerly 2009, Pyron et al. 2015). In addition, the variety of phenotypes we observe in nature is constrained by physical and physiological factors, such that not all phenotypes are viable in nature (Pigliucci 2003, Goswami et al. 2014). Additionally, natural selection shapes phenotypic diversity by favoring traits that give ecological advantages in a certain environment (Kraft et al. 2015, Šímová et al. 2018, Watanabe et al. 2019). On the other hand, the ability of an organism to exhibit many phenotypes in response to environmental cues, known as phenotypic plasticity, can also aid in the development of various phenotypes under diverse environmental conditions (Hendry 2016, Chevin and Hoffmann 2017, Eriksson and Rafajlović 2022). Phenotypic plasticity can help organisms overcome environmental constraints and lead to lineage-wide phenotypic diversification (Valladares et al. 2014, Hendry 2016, Lofeu et al. 2021). Thus, understanding the mechanisms that contribute to the evolution of phenotypic diversity is crucial for predicting how organisms, populations, and communities may adapt to future environmental changes and for understanding the evolutionary history of life on Earth.

Functional traits are often used to infer processes shaping community assembly or the composition of assemblages. It is often assumed that environmental filtering can lead to clustered trait values within communities, whereas competition or niche differentiation can lead to overdispersed trait values (Kraft et al. 2015). These interpretations of community-wide patterns of trait spacing, however, often ignore the influence of processes such as phenotypic plasticity and/or local adaptation, which generate intraspecific phenotypic variation (Messier et al. 2010, Spasojevic et al. 2016). Ignoring within-species trait variation can also lead to underestimating habitat filtering and niche differentiation (Jung et al. 2010, Violle et al. 2012). However, an increasing number of studies are incorporating intraspecific variation to understand the mechanisms operating within species and that are underpinning species assemblages (e.g., (Bolnick et al. 2011, Lepš et al. 2011, Griffiths et al. 2016, Des Roches et al. 2017). This increasing evidence suggests that considering intraspecific trait variation could help bridge the gap between patterns and processes and community structuring. Yet, more research spanning several species and geographical areas is important to test the generality of this link. By gaining a deeper understanding of the processes operating at the community level, it becomes possible to improve our predictions about how climate change and human-caused ecosystem reshaping may influence the structure of communities.

Humans have reshaped ecosystems throughout their existence by converting natural forests into urbanizations or croplands. Human land use frequently results in biodiversity loss, which has detrimental effects on ecosystem functioning and services (Newbold et al. 2015). The rising demand for food production frequently results in land conversion, which forces farmers to switch from traditional, sustainable practices to more intensive ones (Flynn et al. 2009, Beckmann et al. 2019). For example, farmers wishing to increase their crop yield switch to monocultures, which has caused the degradation of natural habitats and ecosystem services (Beckmann et al. 2019). This habitat degradation also modifies the structure of communities, resulting in less stable, less productive, and extinction-prone ecosystems (Cadotte 2007, Cadotte et al. 2011). Developing sustainable practices that mitigate the impact of crop production on biodiversity, improve the sustainability of the landscape, as well as support the livelihood of farmers is becoming increasingly important (Perfecto and Vandermeer 2008, 2010, Iverson et al. 2019). Furthermore, these sustainable practices should be informed by biodiversity assessments that integrate the identity of the species (taxonomic diversity), their evolutionary history (phylogenetic diversity), their potential responses to environmental changes, and the role they play within the habitat (functional diversity). These integrative approaches become necessary to better assess and understand the structure, and dynamics of communities occurring in these modified habitats (Cadotte et al. 2008, 2011, Tucker et al. 2019). Moreover, they highlight that the different facets (taxonomic, phylogenetic, and functional) of diversity are not impacted identically by anthropogenic disturbances (Purschke et al. 2013). For example, Liu et al. (2016) observed a decrease in taxonomic and functional diversity after forest conversion to rubber plantations but found no evidence of changes in phylogenetic diversity. Accordingly, multiple facets of biodiversity could be studied at the same time to better understand how communities, and, in turn, ecosystems, respond to anthropogenic change and to set priorities for conservation planning.

Ants are phenotypically complex organisms found in a diverse range of habitats, and their distribution is influenced by various factors, such as historical events, climate, and human activities. Ants have a sophisticated social structure in which specialized individuals perform specific tasks (e.g., reproduction, brood care, foraging) (Hölldobler and Wilson 1990). Phenotypic diversification in ants is hypothesized to have followed the increase in angiosperm diversity throughout the Late Cretaceous to Early Eocene (Moreau et al. 2006). Despite signs of phylogenetic conservatism, ants continued to diversify over the Miocene to create a wide range of species that are adapted to live in newly emerging arid environments (Andersen 2016, Pie 2016), and some thrive even after anthropogenic influence (Leal et al. 2017, Andersen and Vasconcelos 2022). In several ant lineages, phenotypic diversification was primarily fueled by the expansion of climatic niches (Pie and Traniello 2007, Pie 2016, Economo et al. 2018). Because of the significant compositional and richness variation within ant communities over temperature and/or precipitation gradients (Sanders et al. 2003, Dunn et al. 2009, Fowler et al. 2014), ants make a good model system for studying trait variation under diverse climates. Previous studies have demonstrated that the makeup of ant communities varies along broad environmental gradients (e.g., Retana and Cerdá 2000, Sanders et al. 2003, 2007b, Dunn et al. 2009, Arnan et al. 2012, 2017, Andersen et al. 2015). Although ant phenotypes can differ significantly within and between species and are linked to wide abiotic gradients (Amor et al. 2011, Arnan et al. 2014, Wills et al. 2018, Brassard et al. 2020), little is known about the variation of intraspecific traits (i.e., worker and colony) at the community level. Additionally, anthropogenic disturbance is reshaping ecosystems, making it crucial to investigate how ant lineages and communities react to these changes to create prediction models that guide conservation measures.

1.1 Thesis overview

The goal of my thesis is to investigate the drivers of ant diversity and improve our understanding of the mechanisms behind the observed patterns of biodiversity across natural environmental gradients and the consequences of anthropogenic actions. Together, the chapters in my thesis seek to explore the factors that influence ant diversity and to advance our comprehension of the processes that underlie the patterns of biodiversity that have been observed across natural environmental gradients as well as the results of anthropogenic activities, such as the impacts of climate heterogeneity, trait variation patterns, and management techniques. First, in Chapter 2 of my thesis, I explored the effects of climate heterogeneity on the phenotypic diversity of ants while accounting for their phylogenetic relationships. More specifically, I related two complimentary metrics, phenotypic volume, and phenotypic integration, to the breadth of climatic regimes occupied by different ant lineages to infer the effects of climatic diversity on phenotypic diversity. Then, in Chapter 3, I investigated ant trait variation patterns across organizational levels along an environmental gradient. Given that most work has focused on interspecific trait variation, the processes operating at intraspecific levels have gone undetected. This chapter analyzed the geographic variation of adaptation and plasticity in ant communities at different levels of organization: individual workers (intraspecific), colonies (intraspecific), and species (interspecific). Finally, in Chapter 4, I compared the diversity of ants found in contrasting coffee plantation management strategies and nearby natural vegetation patches. This chapter investigated whether intensive management practices changed and homogenized the taxonomical, phylogenetic, and functional diversity of ants found in coffee plantations. This multi-faceted approach allowed us to go beyond the identity of the species found in each habitat and infer consequences on the evolutionary history and ecosystem services provided by ants in coffee plantations.

My thesis focused on understanding the underlying mechanisms of a hyperdiverse and ubiquitous group of insects and has implications for related fields, as well as for conservation. First, Chapter 2 demonstrates that strongly integrated lineages are limited to specific climate regions and may therefore influence how certain ant lineages may be unable to adapt to environmental change. Then, Chapter 3 shows that combining spatial and community-wide intraspecific trait variation data provides new insights into processes underpinning community structuring along environmental gradients. Afterward, Chapter 4 demonstrates that human activities reshape ecosystems and affect multiple facets of biodiversity. Overall, my thesis shows the importance of accounting for phylogenetic relationships while studying phenotypic diversity and its relationship with the environment to better understand lineage-wide phenotypic constraints. Moreover, it illustrates that integrating multiple organizational levels of trait diversity enables the detection of intraspecific and interspecific mechanisms underlying large-scale diversity patterns of phenotypically complex organisms. Finally, it demonstrates that adopting multi-faceted, large-scale approaches to studying diversity patterns can better inform the underlying mechanisms of biodiversity patterns and better inform conservation efforts and, more specifically, sustainable management strategies.

Chapter 2 Phenotypically integrated lineages could be first to go under global climatic changes

2.1 Abstract

Evolutionary changes in the phenotypes of species composing lineages generated the diversity of life forms in nature. While some lineages tremendously diversified phenotypically when compared to their ancestors, others show stasis. As a result, some lineages are phenotypically diverse while others are much less so. The causes of among-lineage variation in the magnitude of phenotypic diversity, however, remain poorly explored. Owing to evolutionary conservatism, closely related lineages are expected to converge in the magnitude of their phenotypic diversity. However, variation in lineage-wide phenotypic diversity may also arise owing to phenotypic adaptations enabling lineages to expand in novel environmental niches. Here, we test the relative influence of evolutionary conservatism and environmental adaptation on the phenotypic diversity of ant, lineages. Specifically, we measured 10 morphological traits related to foraging strategies, resource use, and thermal regulation on 2,083 ant individuals encompassing 876 species in 86 genera (i.e., lineages). Then, for each genus, we quantified two metrics of phenotypic diversity, namely, phenotypic volume and phenotypic integration. Finally, we examined the relationship between phenotypic diversity and the climatic volume occupied by ant genera, while accounting for species richness and evolutionary relationships among genera. We found that the phenotypic integration of ant genera is negatively related to their occupied climatic volumes, regardless of their evolutionary relationships and species richness. In other words, highly integrated ant genera are confined to specific climatic zones, which could indicate that phenotypic integration prevents colonization and diversification into new climatic zones. These results not only support theories on the evolutionary constraints imposed by phenotypic integration but also suggests that phenotypically integrated lineages are at greater risk of extinction under current scenarios of global climatic change.

2.2 Introduction

The diversity of life forms or phenotypes we observe in nature can be attributed to the emergence of traits throughout the history of extant lineages. The evolution of phenotypic innovations allows species to occupy previously inaccessible ecological states (Miller et al. 2023). While some lineages have tremendously diversified phenotypically when compared to their ancestors, others are more evolutionary conserved (Ackerly 2003, Losos 2008, Pyron et al. 2015). As a result, some lineages are phenotypically diverse while others are much less so (Smith et al. 2014). The causes of among-lineage variation of phenotypic diversity, however, remain poorly explored. Owing to evolutionary conservatism, closely related lineages are expected to exhibit similar degrees of phenotypic diversity (Lord et al. 1995, Watanabe et al. 2019). However, phenotypic diversity in a lineage may also emerge due to evolutionary adaptation enabling persistence in novel environmental niches (Lord et al. 1995, Saenko et al. 2008). Here, we examine the relationship between the phenotypic diversity of ant lineages (i.e., genera) and the climatic volume they occupy globally while accounting for evolutionary relationships.

The phenotypic diversity we observe in a lineage is the outcome of a combination of processes, which include evolutionary conservatism and ecological speciation in response to heterogenous environments (Losos 2008, Rundell and Price 2009, Pyron et al. 2015, Hiller et al. 2019). Evolutionary conservatism means that there is a tendency for ancestral traits to be retained such that closely related species and lineages are expected to share similar traits. If developmental pathways and the constraint they impose on phenotypes are conserved, then the propensity of a lineage to diversify phenotypically should also be conserved (Saenko et al. 2008, Morandin et al. 2016). In this scenario, a phenotypically diverse lineage is most likely to be surrounded by other phenotypically diverse lineages in the evolutionary tree. In addition, phenotypes can evolve throughout the history of a lineage through ecological speciation, which is the response to alterations in selection regimes caused by environmental barriers to gene flow, ultimately influencing genetic and developmental pathways (Schluter 2009, Rundell and Price 2009). Here, exposure to new environments may lead to ecological diversification, which then promotes the expansion of phenotypic trait space occupied by lineages (Raia and Fortelius 2013, Sansalone et al. 2019). The persistence of environmental heterogeneity through time should also favor the persistence of such phenotypic diversity through time (Fournier et al. 2020). For most groups of organisms, however, the relative influence of evolutionary conservatism and environmental adaptation on lineage-wide phenotypic diversity remains mostly unexplored (but see Peixoto et al. 2017, Rodrigues et al. 2019).

Owing to physical and physiological constraints imposed by the natural world, not all combinations of traits are possible, meaning that there are limits to the diversity of phenotypes we can observe in a lineage (Brousseau et al. 2018, Agrawal 2020). The trait combinations describing phenotypic diversity within a lineage can be quantified using one of several metrics (Violle et al. 2007, 2014). Phenotypic volume and phenotypic integration are complementary metrics that can be used to quantify phenotypic diversity at any given level of organization. First, the phenotypic volume represents the multivariate equivalent of the range of trait values along axes of variation (Blonder 2018). Second, phenotypic integration represents a measure of the magnitude of covariance among traits, which can be considered axes of variation (Pigliucci 2003, Houle and Rossoni 2022). This hypothesized that phenotypic integration could limit evolutionary opportunities resulting in a narrower range of trait values observed within a lineage (e.g., Young and Hallgrímsson 2005, Drake and Klingenberg 2007, Bright et al. 2016). However, theoretical models have also suggested that phenotypic integration could facilitate evolutionary change along specific axes or paths of least evolutionary resistance (Goswami et al. 2014, Felice et al. 2018). Furthermore, recent empirical evidence shows that lineages can diversify phenotypically by evolving along paths of least resistance, illustrating how phenotypic integration could enable diversification, as well as lead to convergent shapes across regions and lineages occupying extreme ends of axes of trait variation (Navalón et al. 2020, Hedrick et al. 2020). Though theoretical models suggest that strong integration can facilitate phenotypic evolution, it may also increase vulnerability to selection pressures targeting those axes of variation, therefore, decreasing the range of observed trait values of a lineage (Pigliucci 2003, Houle and Rossoni 2022). Thus, combining phenotypic volume and integration is useful to infer underlying ecological, evolutionary, and historical processes shaping lineage-wide phenotypic diversity (Goswami et al. 2014, Klingenberg 2014, Penna et al. 2017).

Lineage-wide estimates of phenotypic diversity (e.g., volume and integration) can be used to infer the relative influence of evolutionary constraints and environmental adaptation on the phenotypic diversity observed in a lineage. First, tests of phylogenetic signal can be used to infer the strength of conservatism, and whether the propensity of lineages to diversify phenotypically is conserved (Revell et al. 2008, Losos 2008, Houle et al. 2019). While many studies have examined the evolutionary conservatism of phenotypic traits using this approach (e.g., Ackerly 2003, Wiens and Graham 2005, Losos 2008, Peixoto et al. 2017, Rodrigues et al. 2019, Hiller et al. 2019), few have used it to examine evolutionary conservatism in lineage-wide phenotypic diversity (e.g., Lord et al. 1995, Ackerly 2009, Enriquez-Urzelai et al. 2022). Second, one can relate among-lineage variation in phenotypic diversity to the environmental space underlying the distribution of this lineage to infer the role of environmental adaptation. Studies on the relationship between phenotypic diversity and occupied environmental space show that species with integrated phenotypes have a lower trait range (i.e., becoming more specialists) and a more restricted geographical range (e.g., Hermant et al. 2013, Umaña and Swenson 2019, Hughes et al. 2022). However, much less is known about the relationship between lineage-wide phenotypic diversity and occupied climatic space. Thus, studying the phylogenetic and climatic drivers of lineage-wide phenotypic diversity could provide some insight into the evolutionary and environmental forces behind the diversity of life forms observed on Earth (Agrawal 2020).

Ants, an ecologically successful and ubiquitous family of terrestrial invertebrates, are among the most phenotypically diverse organisms on Earth (Fig. 2.1; Kass et al. 2022). Moreau et al. (2006) argue that the rise of angiosperm diversity during the Late Cretaceous to Early Eocene (~80-50 Mya) was a major selective force driving the diversification of ants, who associate with modern plants in multiple ways. Angiosperms provide a wide variety of habitats for ants, which could have promoted phenotypic evolution in many ant lineages (Kaspari and Weiser 1999, Sosiak and Barden 2020). Despite this, many ant traits such as body size and foraging strategies have been shown to be evolutionarily conserved, which may have limited their diversification. Indeed, there is evidence suggesting that ancient ant lineages are associated with conditions of warm temperatures and low seasonality and that many traits related to surviving these conditions are phylogenetically conserved (Donoso 2014, Pie 2016, Arnan et al. 2017). However, historical climatic fluctuations likely resulted in adaptation to new environments and an increase in the phenotypic diversity of some genera (Economo et al. 2018, 2019). For example, ants further diversified during the Miocene (23-5 Mya) into a variety of species adapted to survive in emerging arid biomes such as deserts and tropical savannahs (Dunn et al. 2009, Lessard et al. 2012, Andersen 2016, Andersen and Vasconcelos 2022, Casadei-Ferreira et al. 2022). Thus, the expansion of climatic niches likely drove phenotypical diversification in some ant lineages despite evidence of phylogenetic conservatism (Pie and Traniello 2007, Sarnat and Moreau 2011, Blaimer et al. 2015a, Blanchard and Moreau 2017).

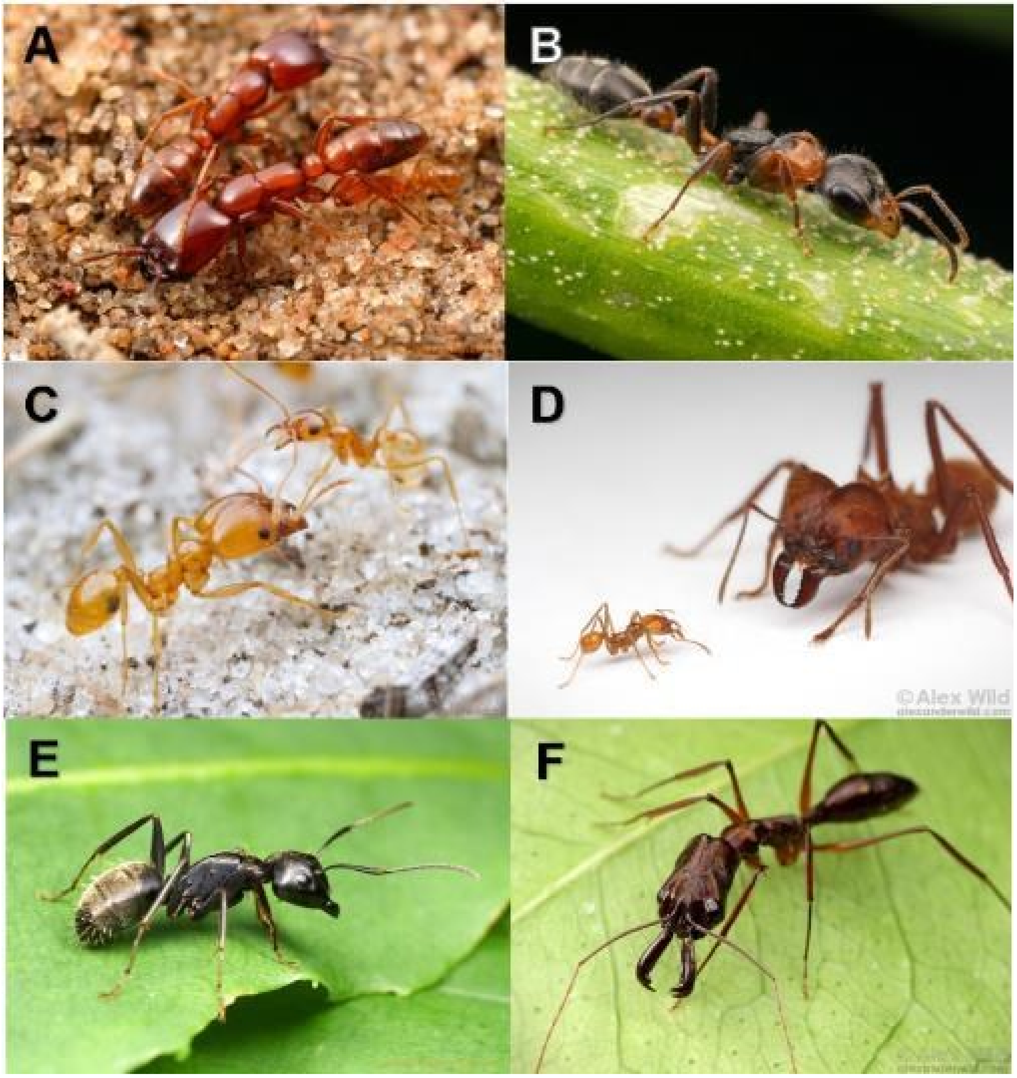


Figure 2.1. Diversity of ant morphologies. A) *Dorylus helvolus*, B) *Pseudomyrmex gracilis*, C) *Pheidole morrisi*, D) *Atta cephalotes*, E) *Camponotus pennsylvanicus*, F) *Odontomachus chelifer*. Photo credit: Alex Wild.

In this study, we investigate how the phenotypic diversity of ant lineages relates to their phylogenetic membership and the breadth of climatic regime they occupy. Specifically, we predict that ant genera occurring in a wide breadth of climate regimes occupy a larger phenotypic volume in morphological space. Additionally, considering that strong trait covariation (phenotypic integration) could limit the range of trait values to a few major axes of variation and constrain trait range (phenotypic volume), certain lineages may not be able to adapt to a diversity of climatic conditions (i.e., limiting occupied environmental space) (Pigliucci 2003, Houle and Rossoni 2022). If environmental conditions select against those few axes of variation, then highly integrated genera should be restricted to specific climatic zones and would be vulnerable to climatic change (Pigliucci 2003, Goswami et al. 2014). Therefore, we expect highly integrated genera to occupy small climatic volumes.

2.3 Methods

2.3.1 Specimen sampling and trait measurement

2.3.1.1 Obtaining species for trait measurement

We obtained trait values from specimen photographs available from the database Antweb (www.antweb.org). We only measured specimens from genera with at least 1 queen specimen imaged and 1 (for monomorphic species) or 3 (for polymorphic species) worker specimens imaged. For each specimen, we measured ten linear traits commonly used by ant ecologists and taxonomists: two measures of head size, four measures of head appendages, two of thorax size, and two of leg length (Table S2.1; Fig. S2.1). To measure these morphological traits from specimen photographs, we used the software ImageJ v1.51j8 (Schneider et al. 2012).

2.3.1.2 Trait measuring

We measured up to four individuals per species: 2 for monomorphic and 4 for polymorphic species. We measured one queen and one worker for each genus (e.g., *Myrmica*, *Ectatomma*, *Lasius*). For polymorphic genera (e.g., *Camponotus*, *Eciton*, *Atta*) we measured queens and minor, intermediate, and major workers. To reduce confounding effects in ant morphology due to environmental variation (Silva and Brandão 2010, Parr et al. 2017, Sosiak and Barden 2020), we measured specimens that occurred within two latitudinal and longitudinal degrees of the imaged queen (Fig. S2.2). When available worker specimens were farther than this, we measured the closest worker specimens to the queen.

Including raw measurements in dimension reduction techniques can result in body size masking other potential contributors to variation (Peres-Neto and Magnan 2004). Thus, we calculated a log-ratio transformation (i.e., Aitchinson's transformation) to account for individual size effects. We used the 'clr' function of the *compositions* package (van den Boogart et al. 2008).

2.3.2 Statistical analyses

2.3.2.1 Visualization of trait correlations and density of observed trait combinations

We performed PCAs to visualize the relationship between traits and the trait space occupied by the measured lineages. For this analysis, we used the log-ratio transformed variables (traits), which we subsequently standardized (z-transformed) to reduce errors due to different measurement scales (Jongman et al. 1995). To visualize trait space occupied by ants, we used the first two PC axes which explained around 75% of the variance to represent a two-dimensional continuum. We used the function ‘princomp’ in R (R Core Team 2022).

To evaluate the occurrence probability of trait combinations (probability contours), we employed kernel density estimations using the ‘kde’ function of the *ks* package for R (Duong 2007). We extracted the contours at 0.5, 0.95, and 0.99 quantiles of the probability distribution in the two-dimensional continuum (i.e., PC1 and PC2). We used the sum of asymptotic mean squared error pilot (i.e., unconstrained bandwidth selector) to avoid infinite contours via the ‘Hpi’ function of the *ks* package (Duong and Hazelton 2003, Duong 2007).

2.3.2.2. Quantifying phenotypic volume and phenotypic integration of ant genera

Data preparation: Given that we wanted to study ant phenotypic volume and phenotypic integration at the genus level, we subset our main dataset to include only genera with three or more species. These subsets were necessary to ensure the phenotypic volume and phenotypic integration is calculated with at least three value points.

Quantifying the volume of the observed trait space using hypervolumes: Hypervolumes can accurately measure the volume of high-dimensional shapes that include holes, disjunctions, or other complex geometrical figures (Hutchinson 1957, Blonder et al. 2014). Additionally, hypervolumes are not as sensitive to outliers (compared to convex hulls, Cornwell et al. 2006). We used the first four PCA axes, which explained at least 80% of the variance, as multidimensional traits for evaluating the n-dimensional hypervolume to reduce dimensions since as dimensionality gets high, stochastic points become sparser, leading to disjunct hypervolumes (Mammola and Cardoso 2020). Using multidimensional trait axes also reduces the correlation between axes and ensures orthogonality (Blonder et al. 2018). We used the one-class support vector machine (SVM) estimation method to build our hypervolumes (Blonder et al.

2014, 2018). In a one-class SVM model, support vectors are used to draw random points uniformly from hyperellipses, which are then resampled and evaluated to retain positively classified points for hypervolume characterization. This estimation method provides a smooth fit around data, is less sensitive to outliers, and provides binary boundary classifications (i.e., in or out). SVM is most appropriate to estimate hypervolume when extreme values in the observed data are thought to represent the boundaries of the data (Blonder et al. 2018). We calculated the observed volume using the 'hypervolume_svm' function of the *hypervolume* package for R (Blonder et al. 2018). The units of the hypervolumes are reported as standard deviations of centered and scaled transformed trait values, raised to the power of the number of dimensions, with higher values representing higher phenotypical volumes.

Quantifying phenotypic integration of measured traits: Overall trait integration can be calculated based on their correlations using the eigenvalue distribution of the correlation matrix. This measure of integration captures the extent to which variance is unequally distributed across principal components or trait axes (Armbruster et al. 2014). Trait integration strength increases because a greater proportion of the total variance is concentrated along fewer principal components or axes (Pavlicev et al. 2009). Specifically, the scaled variance of eigenvalues provides an overall measure of the magnitude of phenotypic integration. A higher variance of eigenvalues can be interpreted as a higher trait integration. We first obtained the correlation matrix using the 'cor' function and then calculated the eigenvalues of this matrix using the 'svd' function. We then calculated the eigenvalue variance using the 'var' function. All functions are available in the *base* package for R (R Core Team 2022).

2.3.2.3. The influence of evolutionary history and environmental variables on phenotypic volume and phenotypic integration

Reconstruction of phylogenetic lineages: To correct for the hypothesized phylogenetic relationships and measure the evolutionary history of the measured species, we used a robust, dated, and ultrametric, genus-level phylogeny available in the literature (Moreau and Bell 2013). We then pruned this tree to only include genera found in our dataset. Subsequently, we used this tree to control for evolutionary relationships among species using phylogenetic generalized least squares (PGLS) described below. In addition, we also used this tree to calculate the phylogenetic signal for phenotypic volume and phenotypic integration using Blomberg's K (Revell et al. 2008, Revell 2010). Blomberg's K lower values indicate a lower phylogenetic signal. The significance of K was evaluated based on comparison with 1000 simulations, creating a null expectation of no phylogenetic signal. K was computed with the function 'phylosig' of the *phytools* package for R (Revell 2012).

Extracting environmental variables: To synthesize the climate diversity where the species of a genus globally occur, we obtained geographical records of the measured species from the Global Ant Biodiversity Informatics project database (GABI; Guénard et al. 2017). Using these coordinates, we then extracted the macroenvironmental variables: annual mean temperature, standard deviation of annual temperature (i.e., temperature seasonality), annual precipitation, and the coefficient of variation of precipitation (i.e., precipitation seasonality) from WorldClim 2.0 (Fick and Hijmans 2017). Following this, we calculated the climatic volume using these four variables, following the same methods described previously to calculate phenotypic volume. A larger climate volume can be interpreted as a specific genus occurring in larger climate diversity. We used the climate volume calculated as a fixed factor in a phylogenetic generalized least squares model to assess the influence of climate diversity on the phenotypic volume and integration of ants.

PGLS model construction: We used a phylogenetic generalized least squares (PGLS) model to account for the phylogenetic non-independence among species in our data and test the effects of climate diversity on phenotypical volume and phenotypic integration (Symonds and Blomberg 2014). Consequently, we used climatic volume and species richness per genus as fixed effects in our model and included either phenotypic volume or integration as our response variables (two separate PGLS models). Climate volume represents the climate diversity occupied by a lineage. We include species richness in our model to account for certain lineages being more speciose than others and to explore the effect of the number of species in our response variables (i.e., phenotypic volume or integration).

PGLS allows the inclusion of the phylogenetic structure of the data as a covariance matrix in a linear model, controlling for phylogenetic autocorrelation (Revell 2010). We assumed Brownian motion to construct the phylogenetic correlation matrix and used maximum likelihood for model parameter estimation. We calculated this model using the 'gls' function of the *nlme* package for R (Pinheiro et al. 2014). All variables were log-transformed before the analysis to improve linearity and heteroskedasticity. To explore the effect of the minimum number of species per genera in our models, we repeated these analyses using subsets of our data including only genera with more than 4 and 5 species (79 and 64 genera, respectively). We then compared these to our main subset of genera including at least 3 species (86 genera, Tables S2.6-S2.8).

2.4 Results

In total, 2391 specimens from 987 species, belonging to 180 genera and 13 subfamilies were measured. Our subset (i.e., considering only genera with 3 species or more), retained 2083 specimens, 876 species, belonging to 86 genera and 10 subfamilies. Of these, 19 genera were dimorphic (i.e., minor, and major workers) and 16 genera were polymorphic (i.e., minor, intermediate, and major workers).

2.4.1 Visualization of trait correlations and trait space occupation

Despite the high morphological diversity in the form and function of ants, there are distinct patterns among trait combinations that define the morphologies of ant lineages. Regarding total trait variation, the first two principal components (PC1 and PC2) explain 75% of variance when all castes are considered, 60% of only queen trait variance, and 83% of minor, 81% of intermediate, and 80% of major worker trait variance (Fig. 2.2, Table S2.2).

The primary axis of differentiation (PC1) integrates eye size across all castes, while the secondary axis (PC2) integrates appendage length (leg and antenna). In general, ants with low PC1 and high PC2 values are generally characterized by large eyes and short appendages, e.g., *Pseudomyrmex* spp, and *Gigantiops* spp, whereas ants with high PC1 and low PC2 values are distinguished by small eyes and long appendages, e.g., *Paratrechina* spp, and *Stigmatoma* spp.

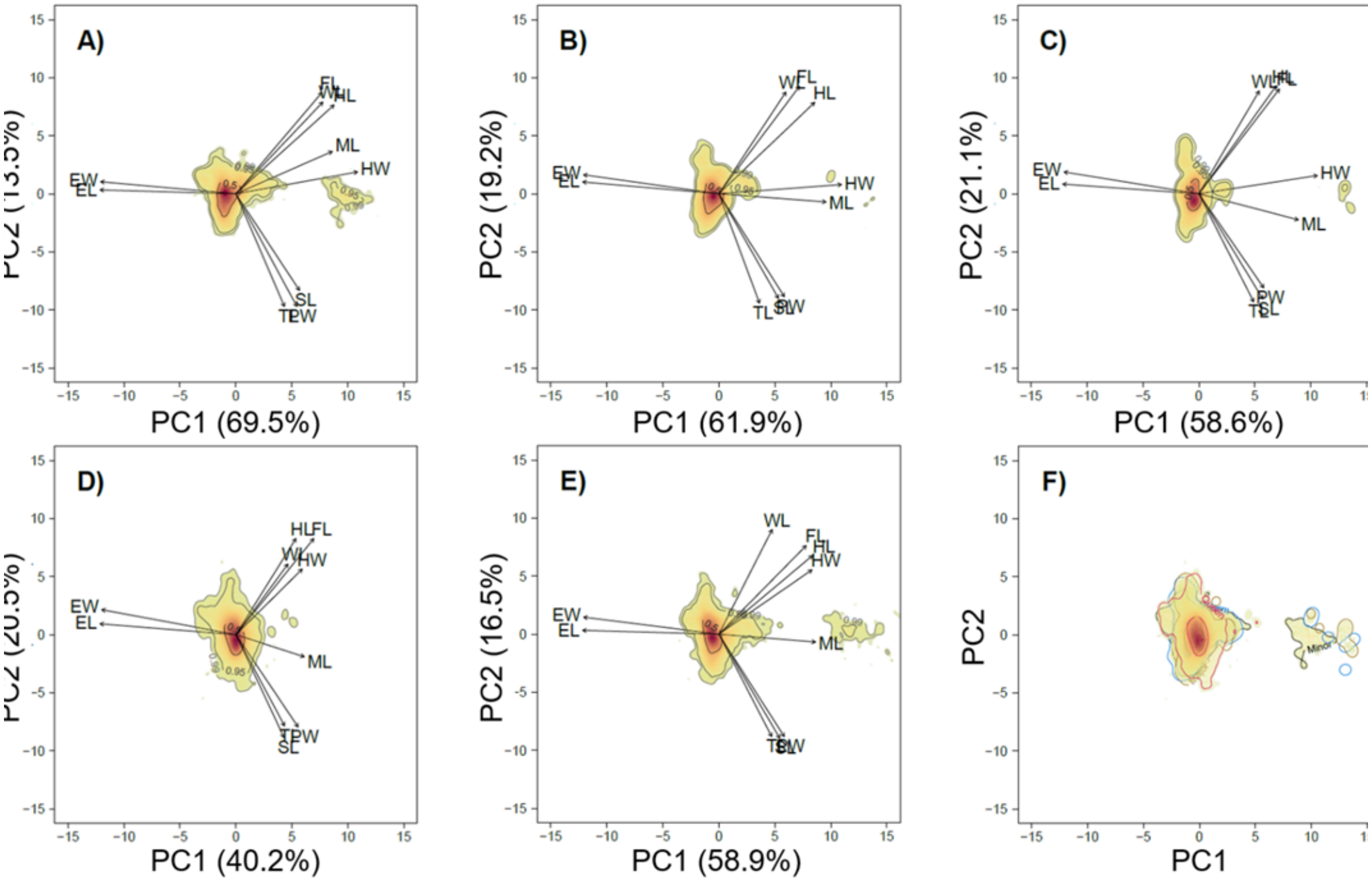


Figure 2.2. PCA biplot depicting the relationships between the measured traits of A) minor, B) intermediate and C) major workers, D) queens, and E) all castes. Arrows indicate the direction and weight of vectors representing the nine traits analyzed: Head Length (HL), Head Width (HW), Mandible Length (ML), Scape Length (SL), Maximum Eye Width (EW), Maximum Eye Length (EL), Body size (Weber's length, WL), Pronotum Width (PW), Hind Femur Length (FL), Hind Tibia Length (TL). Color gradient indicates regions of highest (red) to lowest (light yellow) occurrence probability of species across the morphological space, with contour lines indicating 0.5, 0.95 and 0.99 quantiles. F) Biplot depicting the occupied space of each caste in relation to the others.

2.4.2 Phenotypic volume and phenotypic integration of ants

We found no significant relationship between phenotypic volume and integration (Table S2.4, Fig. S2.6-S2.7). At the genus level, we observed that all genera ranked within the top 10 highest in either volume or integration but not both (Table 2.1). Notably, four genera from the subfamily Myrmicinae: *Cephalotes*, *Pheidole*, *Pogonomyrmex*, and *Strumigenys* ranked in the top 10 highest in volume but ranked low according to phenotypic integration; whereas four genera from the subfamily Ponerinae: *Bothroponera, Neoponera, Platythyrea,* and *Ponera* and one genus from the subfamily Proceratiinae: *Proceratium* ranked in the top 10 highest integrated genera but ranked low according to volume (Table S2.5).

2.4.3 The influence of phylogenetic relationships and climate diversity on phenotypic volume and phenotypic integration

We found a significant negative relationship between climate diversity and phenotypic integration after accounting for phylogenetic relationships but not between climate diversity and phenotypic volume (Fig. 2.3, Table 2.2). In a logarithmic scale, the strength of phenotypic integration decreases by 0.09 units for every unit of occupied climate volume (Table 2.2). Additionally, we found that the number of species is positively related to the phenotypic volume but not to the phenotypic integration of ant lineages. Specifically, on a logarithmic scale, volume increases by 1.43 units for every species in the genus (Table 2.2). Additionally, we did not detect a significant phylogenetic signal for either phenotypic volume ($K = 0.51$, $p = 0.3$) or phenotypic integration ($K = 0.5$, $p = 0.14$, simulations = 1000, Fig. 2.4; Table 2.3). Yet, a caveat to these findings is that we limited our study to genera with at least 3 species, which increased our sample size. When we performed our analyses by limiting our subset to genera with at least 4 or 5 species, our sample size was reduced and, expectedly, some of the observed patterns were no longer significant (Tables S2.6-S2.8).

Table 2.1. Ant genera ranked based of phenotypic volume and integration. Only 19 genera are shown to display the top 10 genera ranked in each category. Full table with 86 genera can be found in Table S2.5.

Subfamily	Genus	Volume	Rank	Phen. Integ.	Rank
Amblyoponinae	*Fulakora*	7.91	9	0.73	13
	Mystrium	5.36	15	0.82	8
	Prionopelta	6.29	12	0.81	10
	Stigmatomma	13.03	5	0.68	15
Dolichoderinae	*Azteca*	7.47	10	0.07	86
Dorylinae	*Cylindromyrmex*	0.54	62	0.94	2
	Dorylus *	13.13	4	0.39	25
	Syscia	0.31	71	0.82	9
Ectatomminae	*Gnamptogenys*	12.10	6	0.60	18
	Typhlomyrmex	0.39	68	0.91	4
Formicinae	*Camponotus* *	9.03	8	0.12	75
Myrmicinae	*Cephalotes* *	19.48	2	0.20	52
	Pheidole *	13.24	3	0.12	77
	Pogonomyrmex	9.16	7	0.13	72
	Strumigenys	29.81	1	0.32	31
Ponerinae	*Bothroponera*	0.14	80	0.83	7
	Neoponera	1.45	41	0.93	3
	Platythyrea	0.23	78	0.87	6
	Ponera	0.76	56	0.97	1
Proceratiinae	*Proceratium*	2.37	30	0.88	5

*Genus contains polymorphic species (i.e., worker caste with distinct phenotypes)

Table 2.2. Phylogenetic Generalized Least Squares model estimates testing relationship between phenotypic volume or phenotypic integration (response variable) and climate volume and species richness per genus (predictor variable) across castes (i.e., queens, and minor, intermediate, and major workers). All models assumed Brownian-motion and parameters estimated using maximum likelihood. Significant predictors (P < 0.05) are highlighted in bold.

	Variable	**Phenotypic Volume**				**Phenotypic Integration**			
		Estimate	**SE**	**t**	**P**	**Estimate**	**SE**	**t**	**P**
All castes *n= 86*	log(ClimVol)	-0.057	0.103	-0.551	0.583	**-0.094**	**0.045**	**-2.076**	**0.042**
	log(SpRich)	**1.433**	**0.255**	**5.619**	**< 0.001**	-0.208	0.112	-1.864	0.067
Queens *n= 86*	log(ClimVol)	0.028	0.14	0.199	0.842	0.016	0.042	0.388	0.699
	log(SpRich)	**2.643**	**0.348**	**7.606**	**< 0.001**	**-0.311**	**0.105**	**-2.968**	**0.004**
Minors *n= 86*	log(ClimVol)	0.116	0.153	0.757	0.452	-0.041	0.034	-1.225	0.225
	log(SpRich)	**2.083**	**0.379**	**5.483**	**< 0.001**	**-0.389**	**0.084**	**-4.648**	**< 0.001**
Intermediates *n= 16*	log(ClimVol)	-0.31	0.189	-1.645	0.126	-0.004	0.085	-0.043	0.966
	log(SpRich)	**3.562**	**0.402**	**8.865**	**< 0.001**	-0.155	0.182	-0.849	0.412
Majors *n= 19*	log(ClimVol)	-0.076	0.271	-0.281	0.783	-0.011	0.045	-0.248	0.808
	log(SpRich)	**2.417**	**0.551**	**4.386**	**< 0.001**	**-0.501**	**0.092**	**-5.472**	**< 0.001**

Table 2.3. Phylogenetic signal of phenotypic volume and phenotypic integration of measured traits. Number of simulations = 1000.

	Phenotypic volume		**Phenotypic integration**	
	K	**p**	**K**	**p**
All castes	0.51	0.3	0.5	0.14
Queens	0.49	0.39	0.46	0.35
Minor workers	0.55	0.38	**0.63**	**< 0.01**
Intermediate workers	0.39	0.94	0.48	0.79
Major workers	0.46	0.85	0.54	0.72

Figure 2.3. Partial residual plots (normalized residuals) of the variability of phenotypic volume (A, B) and phenotypic integration (C, D) explained by climate volume and species richness per genus while accounting for all other variables, including phylogenetic relationships. All variables were log-transformed. See Table 2.2 for the model summary. Blue solid (significant) and dashed (not significant) lines represent the partial residual line of best fit. Red dotted lines represent the fitted LOESS line (best fit).

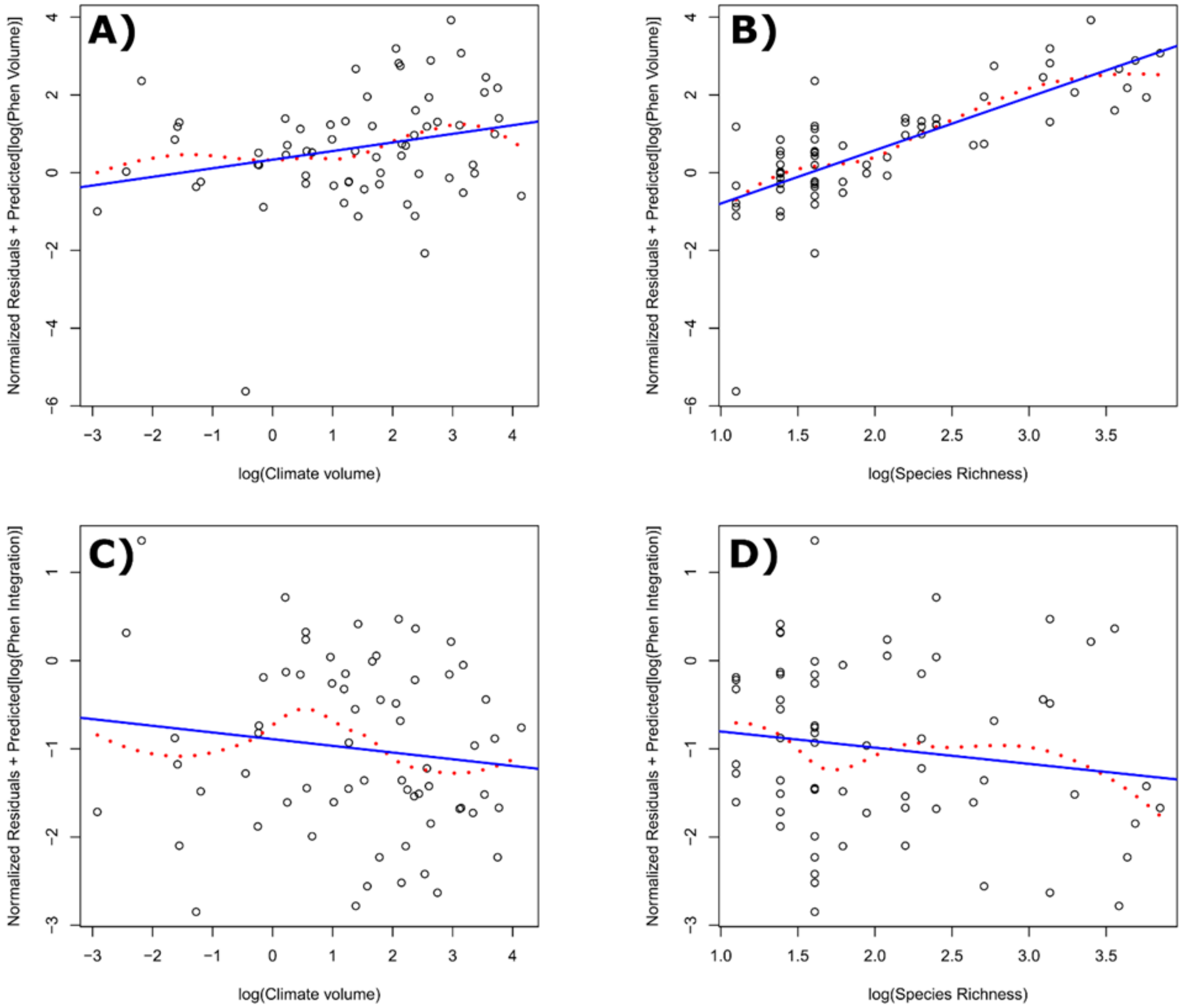


Figure 2.4. Phylogenetic tree of measured ant species (subset from Moreau and Bell 2013). Phenotypic volume (Volume) and phenotypic integration (Phen Int) were calculated including all castes. Volume and integration units have been standardized by subtracting the mean from the observed value and then dividing by the standard deviation for easier visualization.

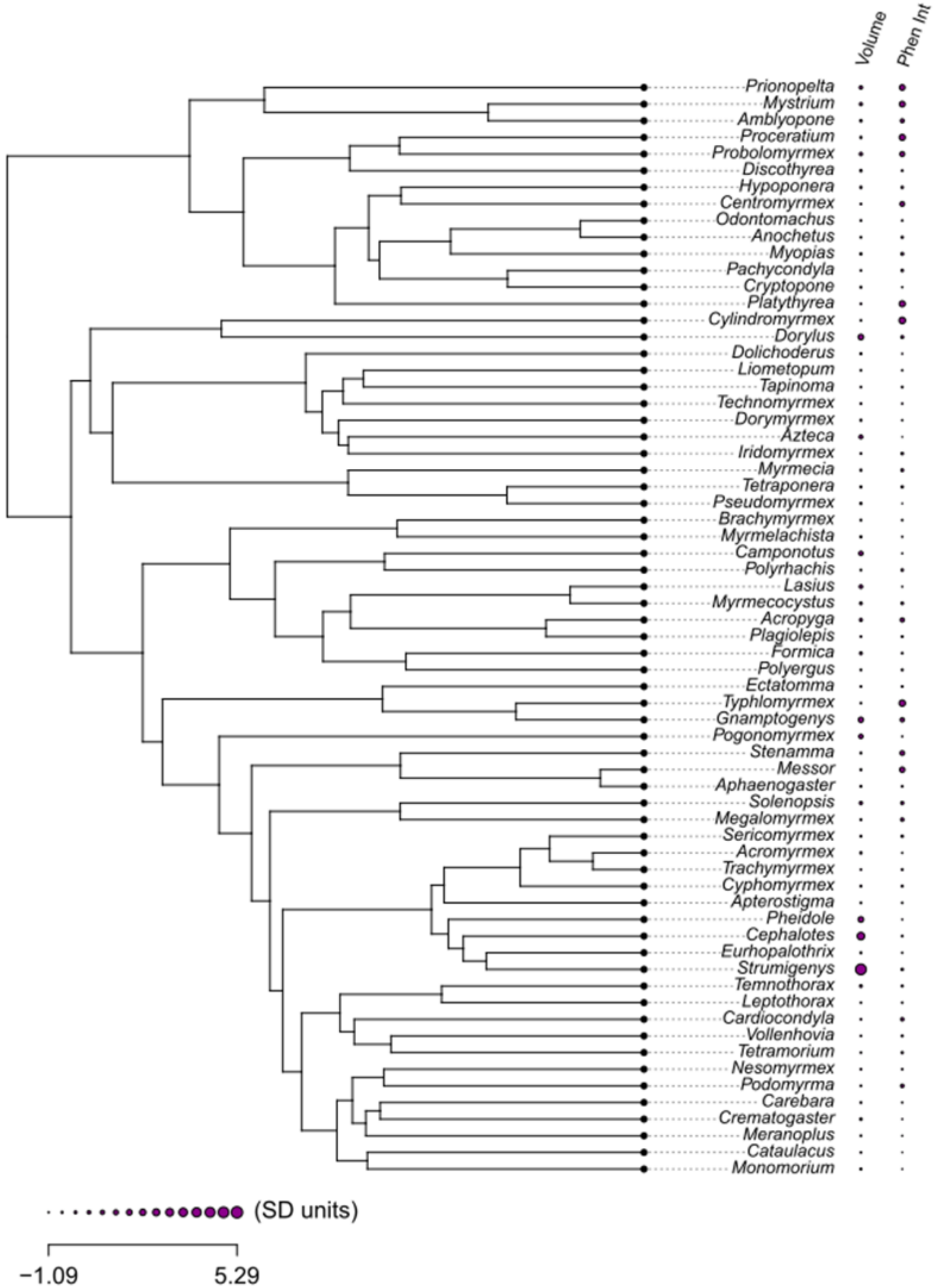
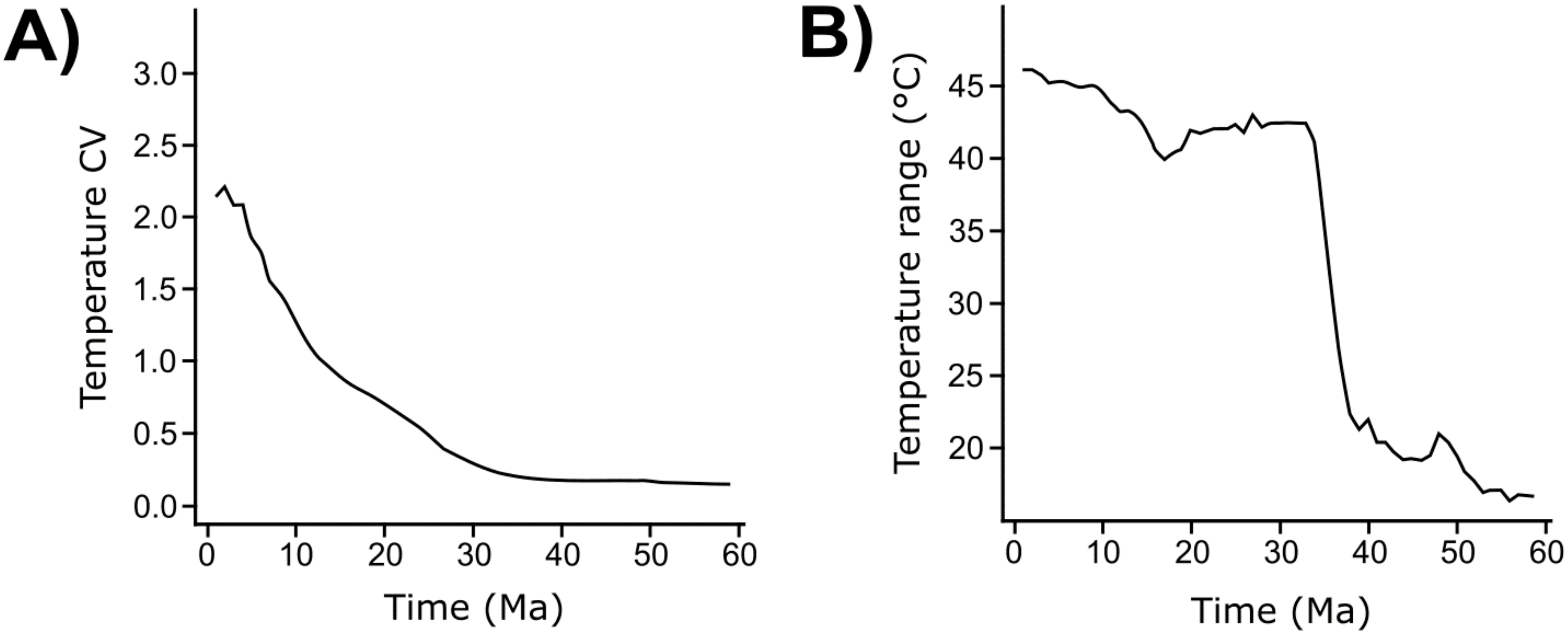


Figure 2.5. Temperature variation expressed as A) the coefficient of variation (CV) and B) temperature range over the last 60 million years in the northern hemisphere. Data from: Hagen et al. (2019)



2.5 Discussion

Our results show that phenotypically integrated lineages are restricted to specific climatic zones. This suggests that phenotypic integration may hamper phenotypic diversification into new climatic zones, both contemporarily and historically. These relationships between phenotypic diversity and occupied climatic volume have implications for the past, present, and future of ant morphological diversity.

We found that strongly integrated ant lineages are restricted to specific climatic zones regardless of evolutionary relationships and the number of species in a lineage. This observation corresponds with theoretical studies proposing that strong integration leads to evolution along a single trajectory, referred to as a line of least evolutionary resistance (Goswami et al. 2014, Felice et al. 2018). Furthermore, this notion aligns with empirical evidence suggesting that strong integration imposes evolutionary constraints, limiting the morphology and adaptability of species (Young and Hallgrímsson 2005, Collar et al. 2014, Rohner and Berger 2023). Indeed, previous work has suggested that high levels of phenotypic integration are associated with narrow habitat distributions (Callahan and Waller 2000, Hermant et al. 2013). Our results further support these notions, indicating that the strongest phenotypically integrated linages are limited to specific climatic zones. Specifically, when analyzing the most integrated genera in our database (Table 2.1) we observe that most highly integrated linages are concentrated in tropical regions (Antmaps.org; Janicki et al. 2016, Guénard et al. 2017). These tropical regions are characterized by relatively stable climates, typified by low temperature and precipitation variability. The significance of stable climates in the context of evolution becomes apparent when considering how large climatic variations can influence niche size and resource availability (Hua and Wiens 2013, Mannion et al. 2014). Hence, stable climates may favor strongly integrated ant lineages, whereas weakly integrated lineages may be able to adapt and respond to environmental change. As a result, strongly integrated lineages could be more vulnerable to the loss of climate diversity and the increased climate unpredictability forecasted by future climate change scenarios (IPCC 2023).

Ant lineages with low degrees of phenotypic integration are found in diverse climate regions, indicating that weak integration has allowed these lineages to adapt to emerging climatic conditions. Ants and other organisms have experienced a significant increase in the global diversity of climate regimes throughout their evolutionary history (Fig. 2.5; Carnaval and Moritz 2008, Calatayud et al. 2019). Specifically, ant phenotypes have evolved over the last ~100 million years, resulting in the diverse array of ant phenotypes observed today (Barden 2017). Yet, higher degrees of phenotypic integration in certain lineages may have limited phenotypic diversification through historical climate changes, confining strongly integrated linages to certain regions of the world (Economo et al. 2018). Considering this, lower degrees of phenotypic integration potentially facilitated the expansion or survival of certain lineages during the formation of extratropical biomes, such as temperate forests and grasslands approximately 40 Mya, while high degrees of integration hindered the expansion of other lineages. However, the lack of a significant relationship between phenotypic volume and climate volume in our study suggests that climate is not the only or main force driving phenotypic expansion in ants. Therefore, we encourage future studies to consider how other factors, such as competition, are related to ant phenotypic volume.

Despite not finding a significant relationship between phenotypic volume and climate diversity occupied by a lineage, some interesting patterns arose when examining the genera that occupy the highest phenotypic (i.e., morphological) volume (Table 2.1, Table S2.4). Notably, lineages that are known for being worker polymorphic occupy large phenotypic volumes and low integration, regardless of their evolutionary relationships. Worker polymorphism may allow for an increased range of trait values in a colony or species (Friedman et al. 2020, Casadei-Ferreira et al. 2022). Furthermore, worker polymorphism creates versatile colonies and species, potentially explaining how ants can thrive in diverse and extreme environments (Wills et al. 2018, La Richelière et al. 2022, Ohyama et al. 2023). Furthermore, our results indicate that patterns of phenotypic diversity are not conserved across ant lineages, potentially resulting in varying evolution rates between lineages and castes. However, the question remains if all ant lineages have the same rate of phenotypic diversification and how trait integration mediates caste differentiation in certain lineages.

Although overall there is no relationship between the phenotypic volume and phenotypic integration of ant genera, we observed that certain ant lineages present similar patterns of phenotypic diversity. Indeed, some ant genera present either a large phenotypic volume or are weakly integrated (Table 2.1, Table S2.5) when all castes are considered together (i.e., as a superorganism) but also when castes are considered individually (Fig. S2.5-S2.7). For example, *Pheidole* (Myrmicinae) and *Camponotus* (Formicinae) are known for being highly diverse, their polymorphism, and ubiquity (Economo et al. 2015, Blaimer et al. 2015b). The combination of high volume and weak integration in these lineages suggests that they have a higher potential of exploring phenotypic space when compared to a strongly integrated lineage (Felice et al. 2018). In contrast, we observe less diverse linages to be strongly integrated. For example, *Ponera* (Ponerinae) and *Proceratium* (Proceratinae) are known for being predators, with some species being specialist predators, and being found mostly in tropical regions (Brown 1979, Schmidt and Shattuck 2014). The combination of low volume and high integration in these lineages suggests that they have limited evolvability and could be susceptible to environmental change (Laughlin and Messier 2015, Felice et al. 2018). In sum, understanding the patterns of phenotypic diversity in ant lineages provides insights into their capacity for adaptation and response to environmental pressures. Finally, these patterns demonstrate that phenotypic volume, and in particular, phenotypic integration, should be considered when studying the effects of environmental change on different lineages.

2.6 Conclusion

Our study represents a first approach to understanding the processes driving ant phenotypic diversity. Even though we found no support for our prediction that lineages occupying a wide breadth of climatic regimes would occupy a larger phenotypic volume, we found support for our second prediction that strongly integrated phenotypes are limited to specific climatic zones.

Our study focused on the lineage-wide morphological diversity of ants which may be related to their ecological success. However, ant ecological strategies also incorporate feeding habits, foraging strategies, and habitat use (Gibb et al. 2023). Likewise, physiological traits have been shown to be important predictors of ant responses to climate change (Diamond et al. 2012, Parr and Bishop 2022). Moreover, Ohyama et al. (2023) recently suggested that queen-worker dimorphism should be measured and included to better understand emergent colony-level traits, which have been shown to vary in response to the environment (Ibarra-Isassi et al. 2021, 2023, Gibb et al. 2023). Therefore, future studies should focus on studying the range and integration of non-morphological, as well as colony-level traits, to gain deeper insights into the mechanisms underlying phenotypic diversity in superorganisms such as ants.

The rapid change of environmental conditions due to climate change threatens to reduce opportunities for maintaining phenotypic variation, with strongly integrated lineages being at risk of climate diversity loss and potentially leading toward lineage-wide phenotypic homogenization. Specifically, these strongly integrated phenotypes may be vulnerable if climatic conditions select against the axis of covariation (Bright et al. 2016). Our study suggests that climate heterogeneity maintains ant phenotypic diversity by supporting climate-specialized, integrated morphologies, regardless of their phylogenetic relationships. Specifically, climate heterogeneity creates a mosaic of habitats and microhabitats, which can support a higher diversity of ant species of multiple lineages. For example, ants found in colder environments tend to be larger and darker (Bishop et al. 2016) and those found in open habitats tend to have longer legs and eyes (Gibb and Parr 2013). Considering that climate change and land use are impacting the distribution and diversity of biomes (Scheffers et al. 2016, Sales et al. 2020), the opportunities to maintain phenotypic variation are becoming more limited. Therefore, a reduced climate diversity threatens less phenotypically diverse lineages, potentially leading toward lineage-wide phenotypic homogenization. The loss of lineages such as specialized predators could render ecosystems less stable, less resilient, and vulnerable to extinction due to the loss of ecosystem functions (Naeem et al. 1994, Oliver et al. 2015, Ibarra-Isassi et al. 2021).

2.7 Supplementary materials

Supplementary Table S2.1. Measured morphological traits and their hypothesized functional significance.

Characteristic	Trait measured	Measurement description	Hypothesized functional significance
Head size	Head length (HL)	The length of the head capsule excluding the mandibles and including clypeus. Measured in frontal view.	Head size has been linked to different ecological strategies with wider heads allowing for larger muscles and stronger mandibles (Kaspari 1993, Sarty et al. 2006, Khalife et al. 2018). Additionally, some ant species block their nest entrance with their heads (Powell et al. 2020).
	Head width (HW)	Maximum head width taken on frontal view. Measured at the widest part of the head excluding the eyes.	
Head appendages	Maximum eye width (MEW)	Maximum eye width measured perpendicular to maximum eye length in profile view.	Eye size is an indicator of navigation, foraging period and substrate, with nocturnal or low-light level foragers having larger eyes (Narendra et al. 2013, Rodrigues and Oliveira 2014).
	Maximum eye length (MEL)	Maximum eye length measured along the maximum diameter in profile view.	
	Mandible length (ML)	Measured as the length of the straight line of the mandible at full closure in frontal view.	Mandible size is linked to foraging strategies, with larger mandibles allowing for larger resource (e.g., prey, leaf, seed) size (Larabee and Suarez 2014, Camargo et al. 2016).
	Scape length (SL)	The maximum straight-line length of the scape excluding the basal constriction. Measured in dorsal, frontal or profile view (longest measurement considered).	Antennal scape length limits the distance ahead of the ant that it receives chemosensory information and is linked to the ability of detecting foraging trails and other chemical signals (Hölldobler and Wilson 1990, Draft et al. 2018).
Thorax size	Weber’s length (WL)	Mesosomal length (or weber's length). Diagonal length of the mesosoma in profile view.	Indicative of overall body size and often linked to resource use, survivability and dispersal (Hood and Tschinkel 1990, Kaspari and Weiser 1999, Wiernasz and Cole 2003, Ness et al. 2004, Yates et al. 2014, Bujan et al. 2016)
	Pronotum width (PW)	The maximum width of the pronotum in dorsal view (spines and tubercles are excluded).	

Characteristic	Trait measured	Measurement description	Hypothesized functional significance
Leg length	Hind femur length (FL)	Straight-line length of hind femur, measured dorsal or profile view (longest measurement considered).	Relates to habitat complexity adaptation, with short legs allowing for greater maneuverability in complex habitats and long legs higher locomotion speed in simpler habitats (Sarty et al. 2006, Gibb and Parr 2013, Yates et al. 2014).
	Hind tibia length (TL)	Straight-line length of hind tibia, measured in dorsal or profile view (longest measurement considered).	

Supplementary Table S2.2. Variable loadings resulting from the principal component analysis (PCA; main text Fig. 2.2). Largest absolute values are bolded. The proportion of variance accounted for a given trait can be obtained by squaring the loading value. Values between -0.09 and 0.09 are not shown due to low PC loading value.

	All castes			**Queens**			**Worker (minor)**		
Prop. of Var.	**PC1**	**PC2**	**PC3**	**PC1**	**PC2**	**PC3**	**PC1**	**PC2**	**PC3**
Trait	**0.589**	**0.165**	**0.088**	**0.402**	**0.205**	**0.125**	**0.695**	**0.136**	**0.062**
Femur length (FL)	0.278	**-0.420**	0.191	0.270	-0.387	0.202	0.272	**-0.484**	0.109
Tibia Length (TL)	0.239	**-0.447**	0.397	0.219	-0.397	0.369	0.235	**-0.525**	0.390
Head Width (HW)	0.326	0.319	-0.232	0.329	0.391	-0.141	0.323	0.366	
Head Length (HL)	0.333	0.269	-0.246	0.264	0.408	-0.228	0.338	0.294	
Eye Length (EL)	**-0.409**			**-0.486**			**-0.378**		
Eye Width (EW)	**-0.408**			**-0.483**			**-0.377**		
Scape Length (SL)	0.266	**-0.438**	-0.223	0.220	**-0.455**	-0.213	0.263	-0.385	-0.476
Mandible Length (ML)	0.291		**-0.535**	0.249		**-0.602**	0.286	0.121	**-0.663**
Pronotum Width (PW)	0.242	**0.458**	0.443	0.223	0.292	0.455	0.314	0.319	0.334
Body size (WL)	0.317	0.211	0.398	0.270	0.257	0.364	0.342		0.227

	Worker (intermediate)			**Worker (major)**		
Prop. of Var.	**PC1**	**PC2**	**PC3**	**PC1**	**PC2**	**PC3**
Trait	**0.619**	**0.192**	**0.071**	**0.586**	**0.211**	**0.088**
Femur length (FL)	0.266	-0.403		0.257	-0.360	0.245
Tibia Length (TL)	0.188	**-0.489**	0.511	0.234	**-0.445**	0.325
Head Width (HW)	0.312	0.402		0.311	0.390	-0.165
Head Length (HL)	0.339	0.312		0.304	0.409	-0.175
Eye Length (EL)	**-0.399**			**-0.411**		
Eye Width (EW)	**-0.399**			**-0.409**		
Scape Length (SL)	0.249	**-0.423**	-0.229	0.262	**-0.415**	-0.249
Mandible Length (ML)	0.314		**-0.643**	0.306		**-0.615**
Pronotum Width (PW)	0.269	0.395	0.472	0.249	0.409	0.488
Body size (WL)	0.360		0.202	0.360		0.312

Supplementary Table S2.3. Mean and standard deviation (SD) of phenotypic volume and phenotypic integration of ant castes. Phenotypic volumes are reported as means (±SD) of standard deviations of the multidimensional trait axes, raised to the power of the number of dimensions (higher values indicate larger volumes). Phenotypic integration is reported as means (±SD) of the scaled variance of eigenvalues of the correlation matrix (higher values indicate stronger integration). Values below the title represent the means (±SD) per caste. Subfamilies are sorted from oldest to more recent clade.

	All Castes		***Queens***		***Minor workers***		***Interm. workers***		***Major workers***	
Subfamily	**Phen. Vol. (3.0±4.7)**	**Phen. Int. (0.3±0.3)**	**Phen. Vol. (1.1±2.6)**	**Phen. Int. (0.3±0.2)**	**Phen. Vol. (0.4±1.3)**	**Phen. Int. (0.4±0.3)**	**Phen. Vol. (0.5±0.7)**	**Phen. Int. (0.4±0.2)**	**Phen. Vol. (0.3±0.4)**	**Phen. Int. (0.4±0.2)**
Amblyoponinae	7.22±3.61	0.71±0.11	0.38±0.43	0.26±0.09	0.8±0.51	0.84±0.14	0.02±0.02	0.73±0.11	0.43±0.41	0.73±0.34
Dolichoderinae	1.86±2.56	0.17±0.08	1.01±1.9	0.26±0.19	0.2±0.32	0.3±0.16	0.49±0.69	0.31±0.24	0.28±0.39	0.31±0.23
Dorylinae	4.66±7.34	0.72±0.29	0.02±0.03	0.58±0.23	0.25±0.26	0.88±0.13				
Ectatomminae	4.3±6.76	0.57±0.35	5.05±8.72	0.57±0.26	0.53±0.88	0.51±0.42				
Formicinae	2.79±2.55	0.25±0.19	1.54±2.24	0.29±0.18	0.26±0.43	0.33±0.23	0.7±0.91	0.34±0.23	0.27±0.42	0.31±0.16
Myrmeciinae	2.01[a]	0.37[a]	1.08[a]	0.49[a]	0.005[a]	0.3[a]				
Myrmicinae	3.23±6.13	0.25±0.16	1.25±2.75	0.28±0.15	0.69±1.95	0.33±0.18	0.42±0.75	0.37±0.13	0.26±0.49	0.34±0.25
Ponerinae	1.02±0.92	0.52±0.31	0.1±0.14	0.35±0.13	0.1±0.17	0.51±0.28				
Proceratiinae	3.76±1.86	0.6±0.34	0.44±0.62	0.28±0.08	0.3±0.27	0.48±0.24				
Pseudomyrmecinae	2.16±0.51	0.19±0.15	1.96±1.46	0.37±0.36	0.19±0.11	0.18±0.06				

[a] Only one genus of Myrmeciinae included more than 3 species.

Supplementary Table S2.4. Summary of the linear models analyzing the relationship between phenotypic volume and integration. All models included log-scaled phenotypic volume as the response variable and log-scaled phenotypic integration as the predictor variable.

	R^2	**Intercept**	**Slope**	**P value**
All castes	0.001	0.22	0.004	0.984
Queens	0.039	-3.098	-0.844	0.067
Minors	0.001	-2.806	-0.126	0.744
Intermediates	0.04	-4.096	-1.026	0.456
Majors	0.153	-5.444	-1.693	0.098

Supplementary Table S2.5. Ant genera ranking based on volume and phenotypic integration when all castes are considered.

Subfamily	Genus	All castes			
		Volume	**Rank**	**Phen. Int.**	**Rank**
Amblyoponinae	*Amblyopone*	3.54	22	0.54	20
	Fulakora	7.91	9	0.73	13
	Mystrium	5.36	15	0.82	8
	Prionopelta	6.29	12	0.81	10
	Stigmatomma	13.03	5	0.68	15
Dolichoderinae	*Azteca*	7.47	10	0.07	86
	Dolichoderus	1.85	36	0.12	74
	Dorymyrmex	1.92	35	0.15	63
	Iridomyrmex	0.49	64	0.33	30
	Liometopum	0.50	63	0.16	62
	Tapinoma	0.26	75	0.21	49
	Technomyrmex	0.56	61	0.15	64
Dorylinae	*Cylindromyrmex*	0.54	62	0.94	2
	Dorylus	13.13	4	0.39	25
	Syscia	0.31	71	0.82	9
Ectatomminae	*Ectatomma*	0.41	67	0.21	48
	Gnamptogenys	12.10	6	0.60	18
	Typhlomyrmex	0.39	68	0.91	4
Formicinae	*Acropyga*	4.86	16	0.58	19
	Brachymyrmex	1.11	49	0.19	54
	Camponotus	9.03	8	0.12	75
	Cladomyrma	0.62	59	0.13	73
	Colobopsis	1.65	38	0.12	79
	Echinopla	0.80	54	0.10	82
	Formica	4.31	17	0.21	50
	Lasius	6.73	11	0.12	78
	Myrmecocystus	4.09	19	0.34	29
	Myrmelachista	2.26	31	0.14	69
	Nylanderia	2.89	24	0.15	65
	Paraparatrechina	1.38	43	0.76	11
	Plagiolepis	0.29	73	0.23	45
	Polyergus	0.93	52	0.24	42
	Polyrhachis	0.84	53	0.32	34
Myrmeciinae	*Myrmecia*	2.01	33	0.37	27
Myrmicinae	*Acromyrmex*	2.62	27	0.14	71
	Adelomyrmex	3.93	20	0.11	80

	Aphaenogaster	1.34	46	0.21	51
	Apterostigma	0.67	57	0.17	61
	Cardiocondyla	0.22	79	0.46	23
	Carebara	0.94	51	0.15	66
	Cataulacus	0.26	77	0.08	85
	Cephalotes	19.48	2	0.20	52
	Crematogaster	3.76	21	0.11	81
	Cyphomyrmex	1.08	50	0.24	44
	Eurhopalothrix	1.38	44	0.15	68
	Leptothorax	0.10	82	0.18	58
	Megalomyrmex	1.19	47	0.47	22
	Meranoplus	0.57	60	0.12	76
	Messor	1.48	40	0.76	12
	Monomorium	2.00	34	0.10	83
	Mycetophylax	0.10	81	0.20	53
	Myrmica	1.40	42	0.37	28
	Nesomyrmex	0.31	72	0.18	56
	Octostruma	2.51	29	0.18	55
	Pheidole	13.24	3	0.12	77
	Podomyrma	0.45	65	0.47	21
	Pogonomyrmex	9.16	7	0.13	72
	Rogeria	0.06	84	0.18	59
	Sericomyrmex	1.15	48	0.31	36
	Solenopsis	5.56	14	0.42	24
	Stenamma	1.35	45	0.65	17
	Strumigenys	29.81	1	0.32	31
	Syllophopsis	0.28	74	0.26	40
	Temnothorax	4.27	18	0.26	41
	Tetramorium	0.26	76	0.27	39
	Trachymyrmex	0.02	86	0.23	46
	Veromessor	2.11	32	0.15	67
	Vitsika	0.05	85	0.17	60
	Vollenhovia	0.07	83	0.24	43
Ponerinae	*Anochetus*	0.79	55	0.28	38
	Bothroponera	0.14	80	0.83	7
	Centromyrmex	0.36	70	0.66	16
	Cryptopone	1.55	39	0.14	70
	Hypoponera	2.66	26	0.31	35
	Myopias	2.86	25	0.38	26
	Neoponera	1.45	41	0.93	3
	Odontomachus	0.67	58	0.18	57

	Pachycondyla	0.38	69	0.32	32
	Platythyrea	0.23	78	0.87	6
	Ponera	0.76	56	0.97	1
	Rasopone	0.45	66	0.32	33
Proceratiinae	*Discothyrea*	3.03	23	0.22	47
	Probolomyrmex	5.87	13	0.69	14
	Proceratium	2.37	30	0.88	5
Pseudomyrmecinae	*Pseudomyrmex*	2.52	28	0.09	84
	Tetraponera	1.80	37	0.29	37

Supplementary Table S2.6. Summary of the slope obtained from the Phylogenetic Generalized Least Squares models using subsets of 5, 4 and 3 species per genus and testing relationship between phenotypic volume or phenotypic integration (response variables) and climate volume and species richness per genus (predictor variables) across castes (i.e., queens, and minor, intermediate, and major workers). All models assumed Brownian-motion and parameters estimated using maximum likelihood. Significant predictors (P < 0.05) are highlighted in bold.

	Variable	**5 spp per genus**		**4 spp per genus**		**3 spp per genus**	
		Volume	**PhenInt**	**Volume**	**PhenInt**	**Volume**	**PhenInt**
All castes	log(ClimVol)	-0.152	0.119	-0.135	**-0.106**	-0.057	**-0.094**
	log(SpRich)	**1.243**	**-0.33**	**1.255**	**-0.182**	**1.433**	-0.208
Queens	log(ClimVol)	-0.026	0.002	0.018	0.009	0.028	0.016
	log(SpRich)	**1.899**	**-0.312**	**2.083**	**-0.284**	**2.643**	**-0.311**
Minors	log(ClimVol)	0.061	-0.019	0.01	-0.036	0.116	-0.041
	log(SpRich)	**1.622**	**-0.349**	**1.762**	**-0.338**	**2.083**	**-0.389**
Intermediates	log(ClimVol)	-0.288	-0.023	-0.255	-0.123	-0.31	-0.004
	log(SpRich)	**3.132**	-0.205	**3.224**	-0.173	**3.562**	-0.155
Majors	log(ClimVol)	**-0.531**	-0.026	-0.442	-0.041	-0.076	-0.011
	log(SpRich)	**2.324**	**-0.376**	**2.581**	**-0.43**	**2.417**	**-0.501**

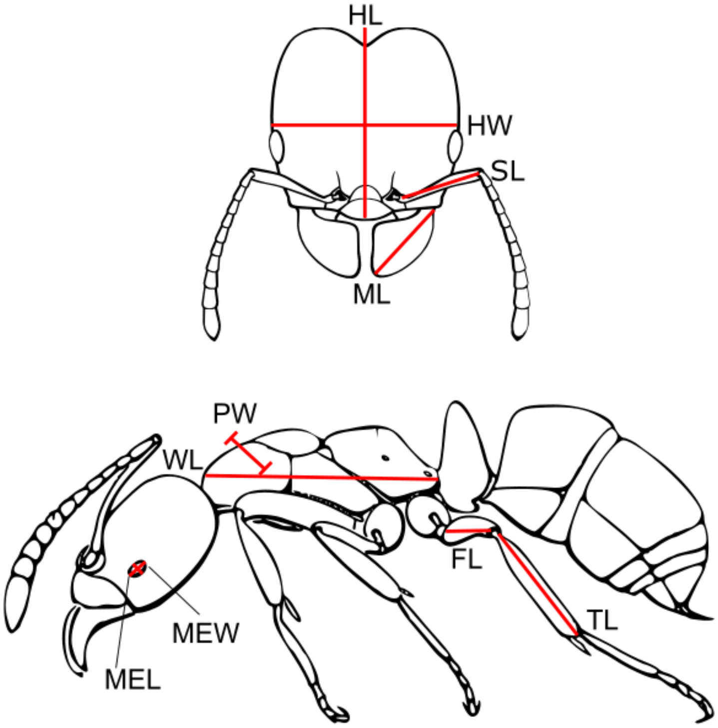
Supplementary Table S2.7. Summary of phylogenetic signal analyses of phenotypic volume of measured traits using subsets of 5, 4 and 3 species per genus. Number of simulations = 1000.

	Phenotypic volume					
	5 spp per genus		**4 spp per genus**		**3 spp per genus**	
	K	**p**	**K**	**p**	**K**	**p**
All castes	0.65	0.08	0.56	0.26	0.51	0.3
Queens	0.64	0.14	0.59	0.27	0.49	0.39
Minor workers	0.66	0.14	0.60	0.27	0.55	0.38
Intermediate workers	0.75	0.67	0.86	0.37	0.39	0.94
Major workers	0.79	0.42	0.77	0.38	0.46	0.85

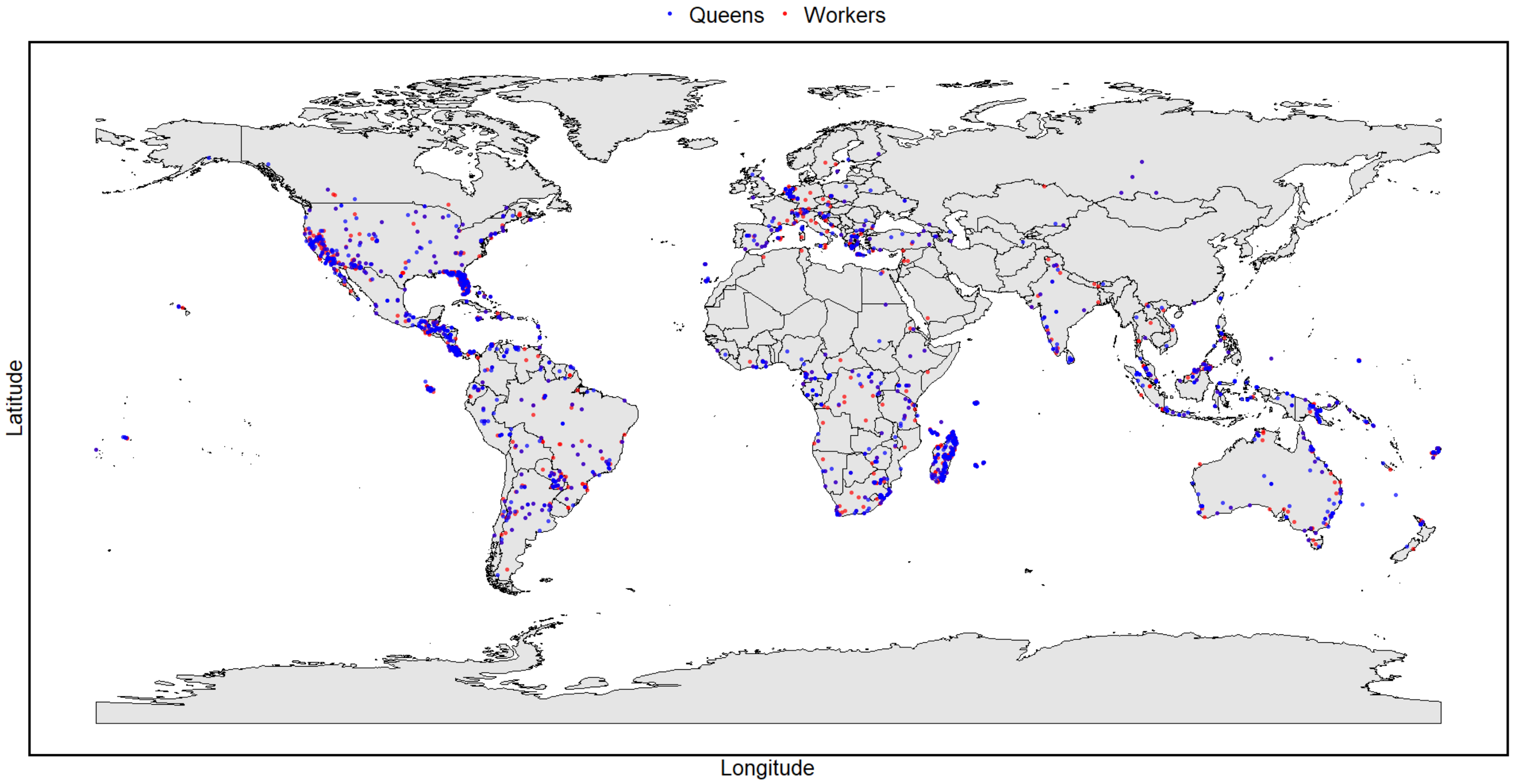
Supplementary Table S2.8. Summary of phylogenetic signal analyses of phenotypic integration of measured traits using subsets of 5, 4 and 3 species per genus. Number of simulations = 1000.

	Phenotypic integration					
	5 spp per genus		**4 spp per genus**		**3 spp per genus**	
	K	**p**	**K**	**p**	**K**	**p**
All castes	0.37	0.82	0.45	0.53	0.5	0.14
Queens	0.59	0.09	0.46	0.51	0.46	0.35
Minor workers	0.48	0.45	**0.66**	**0.02**	**0.63**	**< 0.01**
Intermediate workers	1.08	0.14	0.75	0.64	0.48	0.79
Major workers	0.66	0.51	0.65	0.58	0.54	0.72

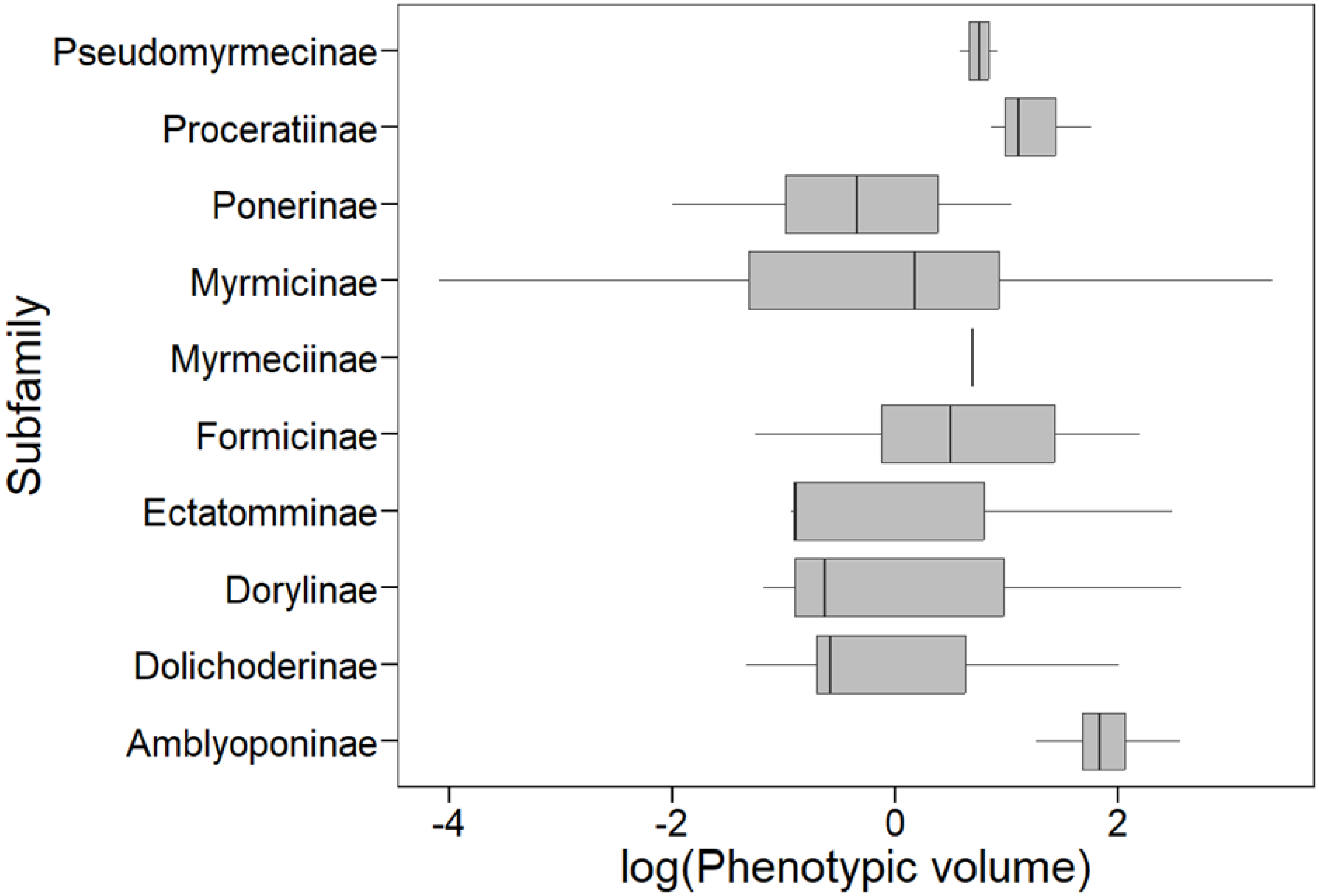
Supplementary Figure S2.1. Diagram of morphometric measurements used in this study. HL = Head Length; HW = Head Width; ML = Mandible Length; SL = Scape Length; MEW = Maximum Eye Width; MEL = Maximum Eye Length; WL = Weber's length; PW = Pronotum Width; FL= Hind Femur Length; TL = Hind Tibia Length. Measurement descriptions can be found in Table S2.1.



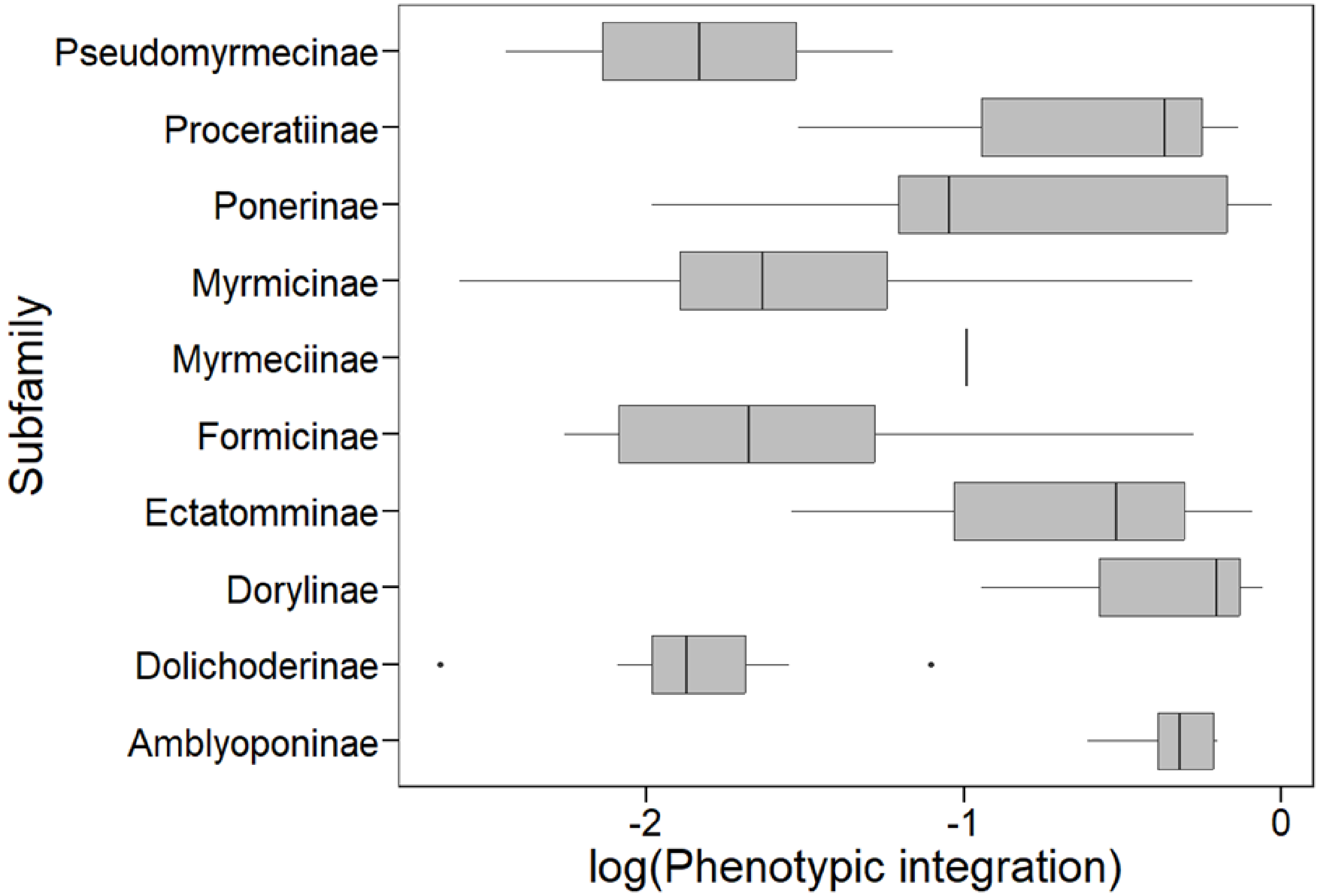
Supplementary Figure S2.2. Geographic distribution of measured specimens. Blue points represent queen specimens and red points represent worker specimens.



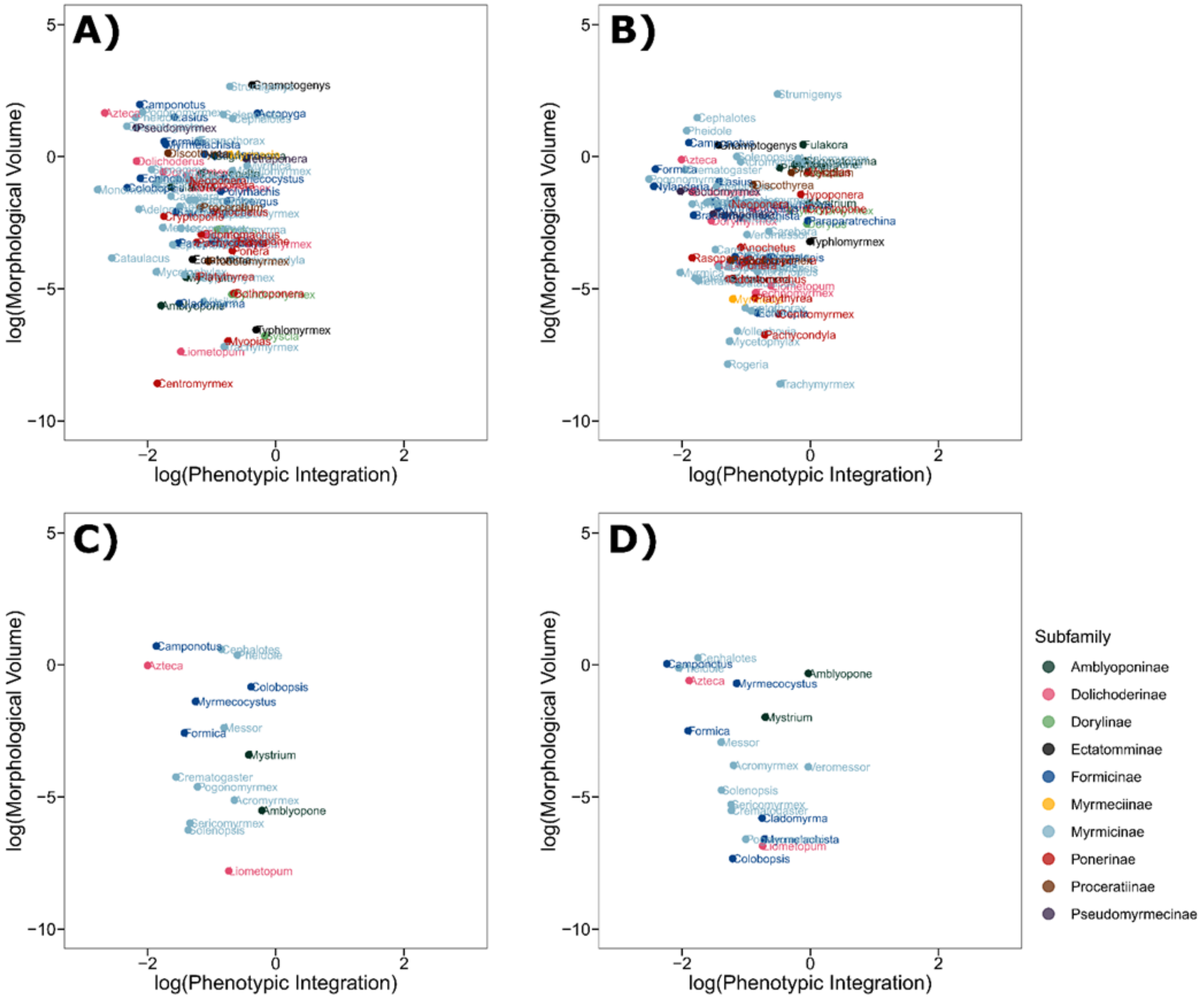
Supplementary Figure S2.3. Boxplot of phenotypic volume (log-scaled) per ant subfamily.



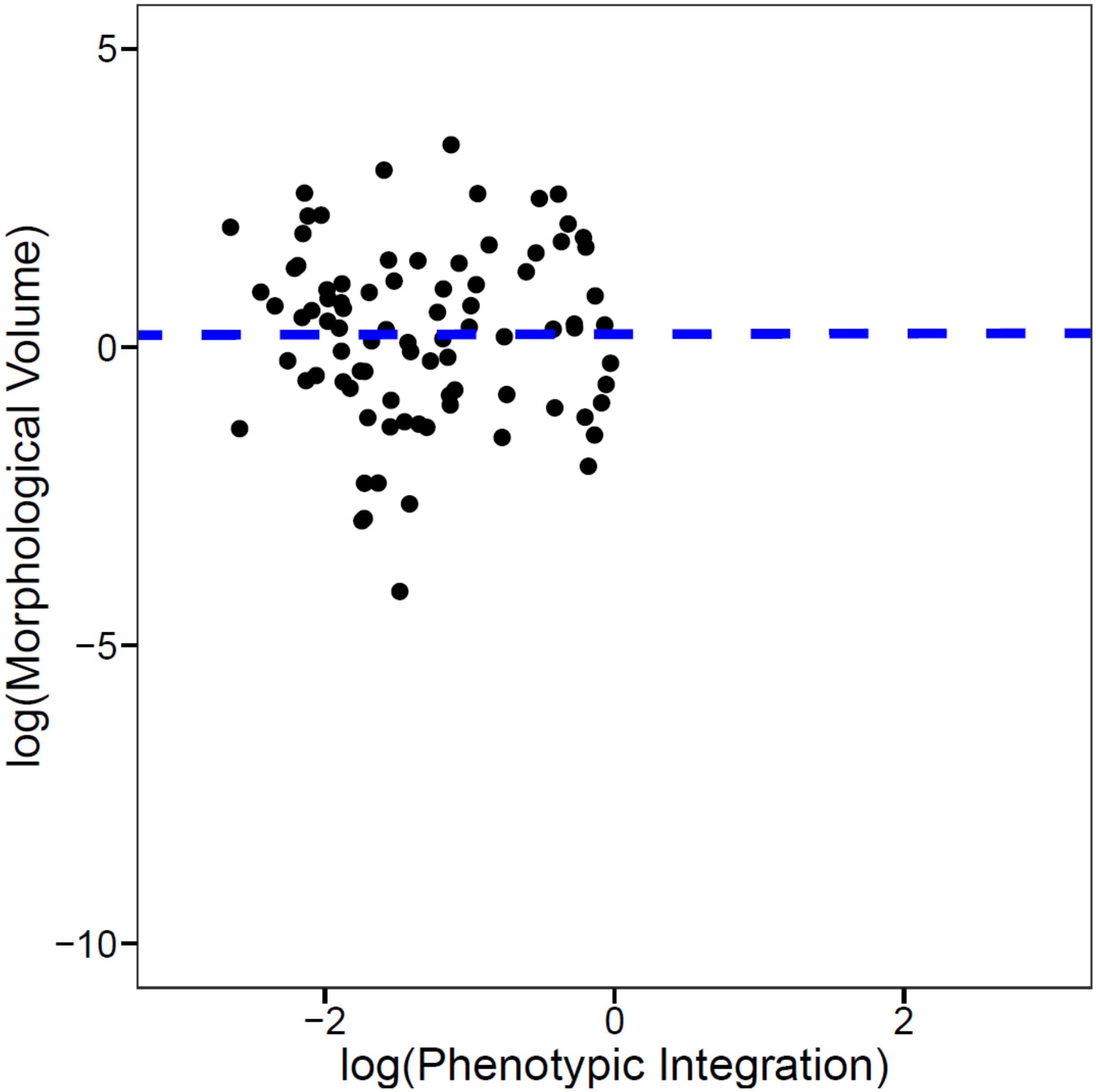
Supplementary Figure S2.4. Boxplot of phenotypic integration (log-scaled) per ant subfamily.



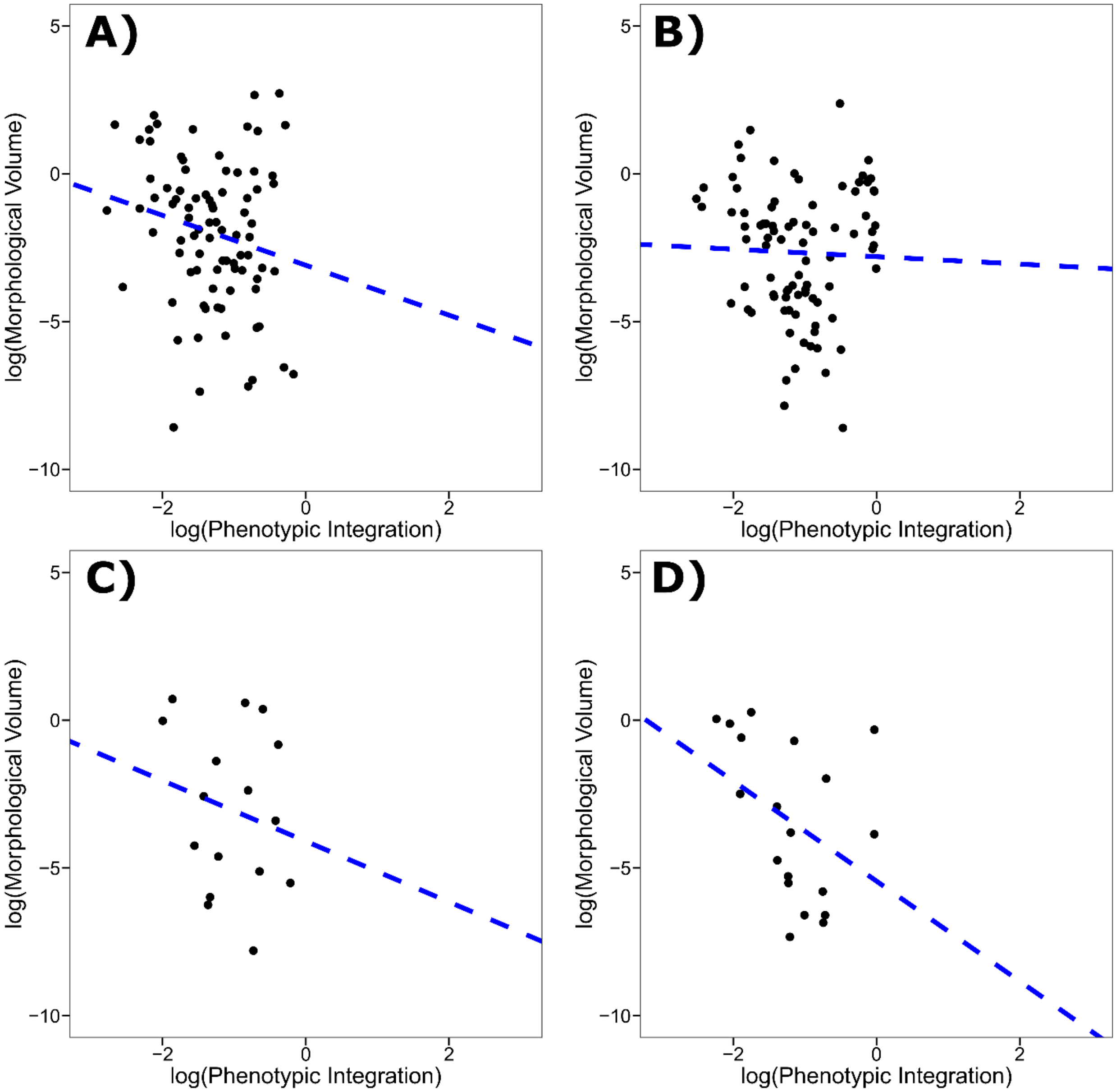
Supplementary Figure S2.5. Scatterplot of strength of phenotypic integration and phenotypic (i.e., morphological) volume determined by the measured traits of (A) queens, (B) minor, (C) intermediate, and (D) major workers. Colored points represent subfamilies.



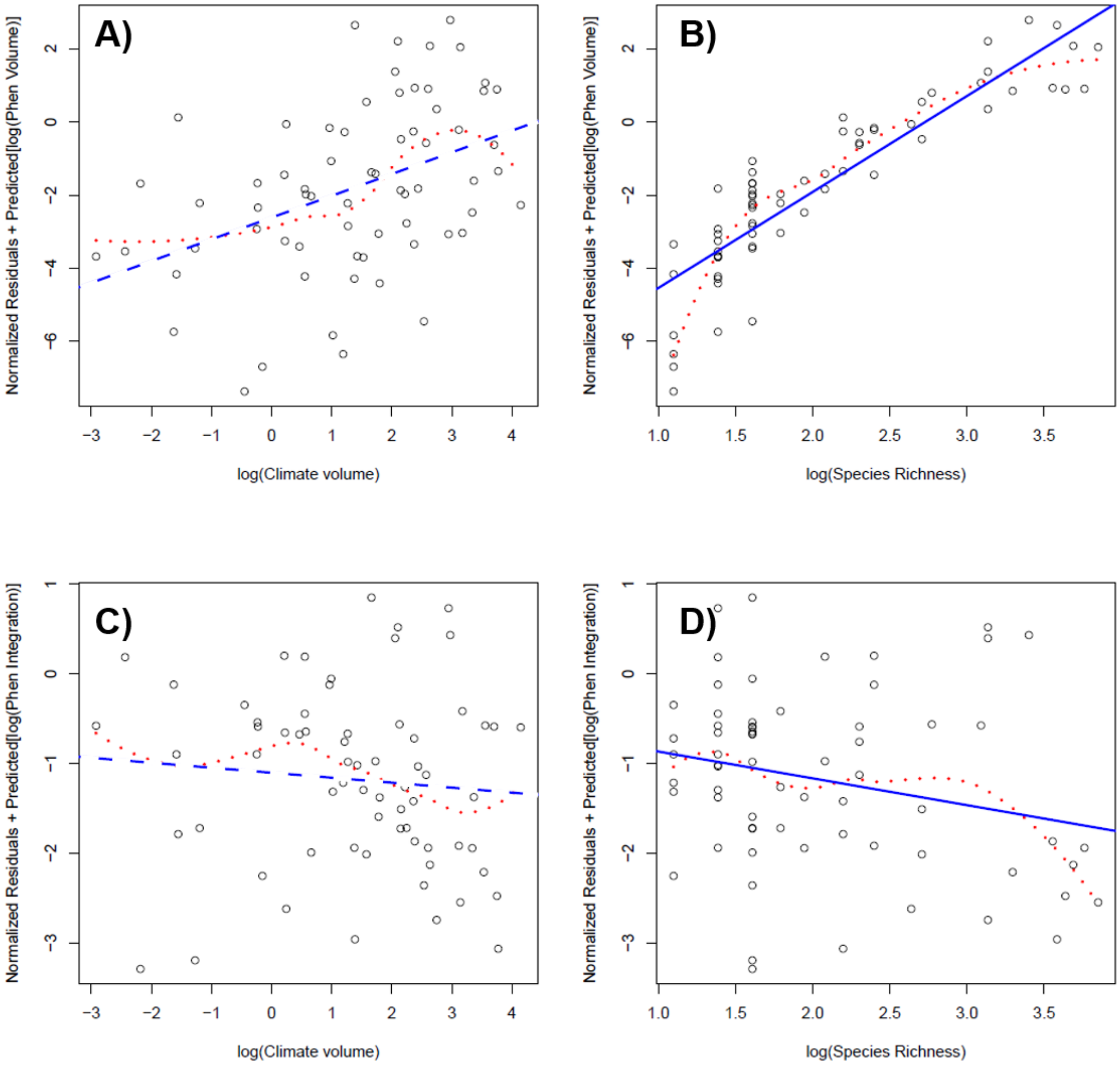
Supplementary Figure S2.6. Scatterplot of strength of phenotypic integration and phenotypic (i.e., morphological) volume determined by the measured traits of all castes combined. Black circles represent genera. Blue dashed line represents a not significant trendline. See Table S2.4 for model summary.



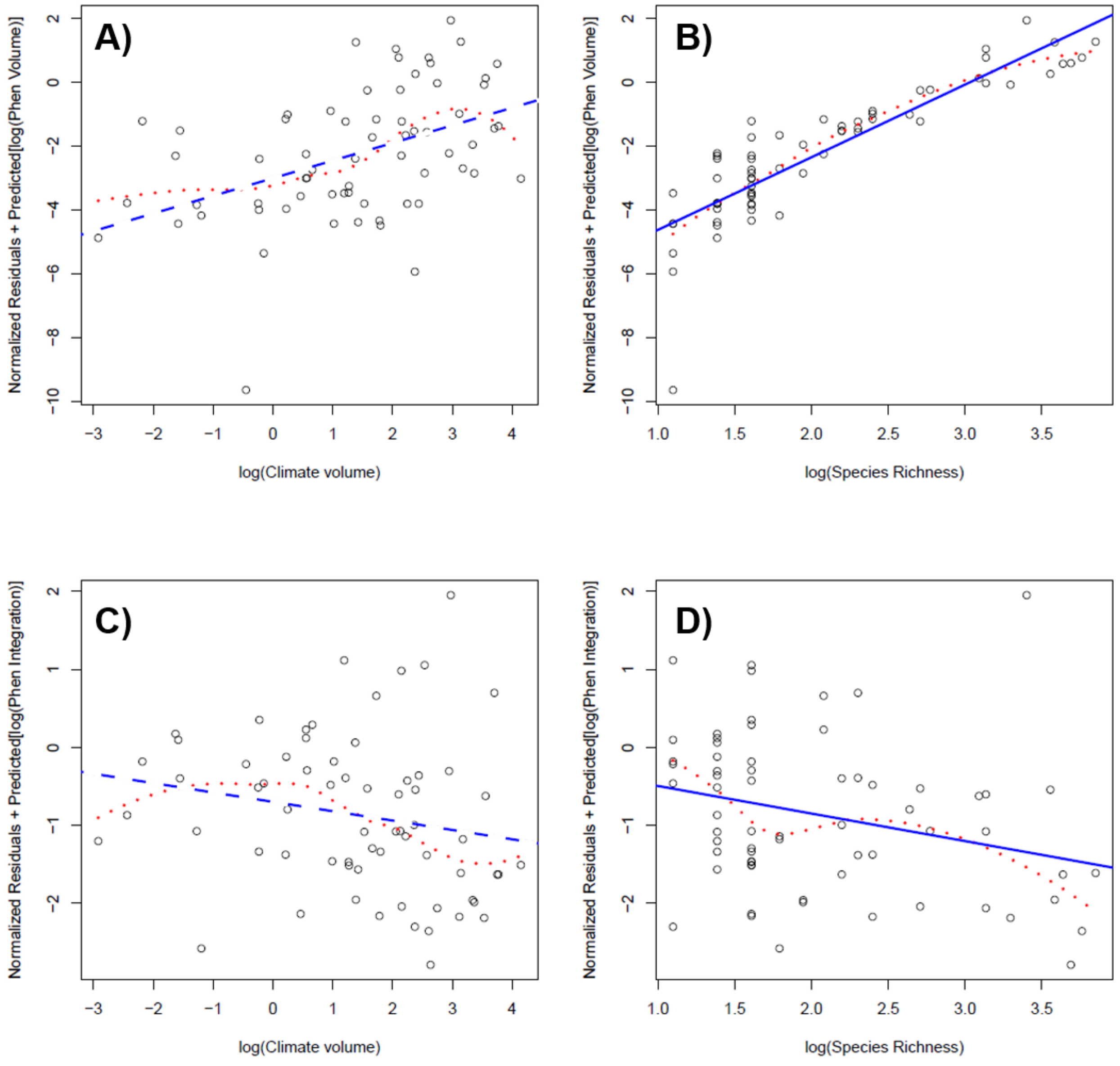
Supplementary Figure S2.7. Scatterplot of strength of phenotypic integration and phenotypic (i.e., morphological) volume determined by the measured traits of (A) queens, (B) minor, (C) intermediate, and (D) major workers. Black circles represent genera. Blue dashed line represents a not significant trendline. See Table S2.4 for model summary.



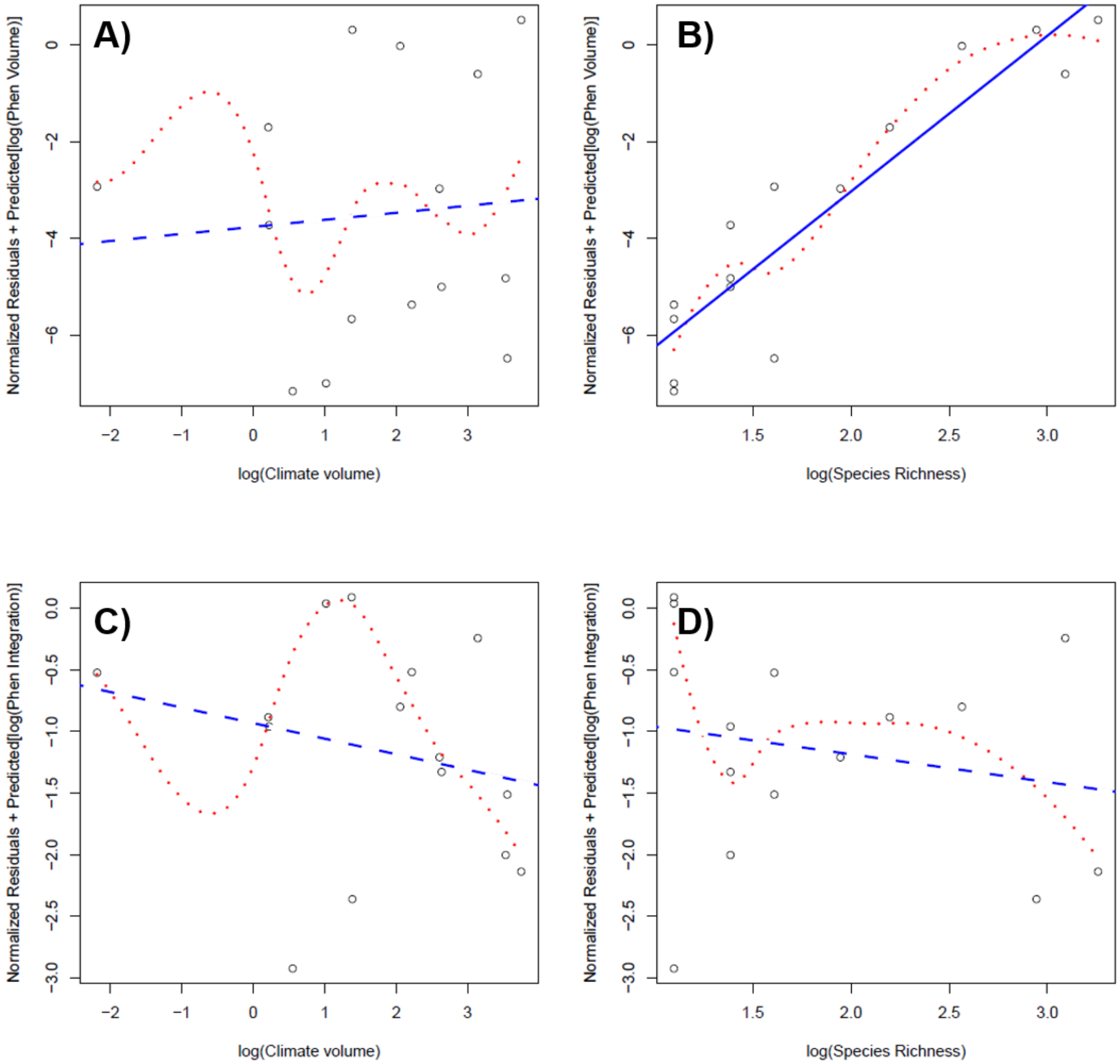
Supplementary Figure S2.8. Partial residual plots (normalized residuals + dependent variable) of the variability of queen's phenotypic volume (A, B) and phenotypic integration (C, D) explained by climate volume and species richness per genus while accounting for phylogenetic relationships (using PGLS). All variables were log-transformed. See Table 2.2 for the model summary. Blue solid (significant) and dashed (not significant) lines represent the partial residual line of best fit. Red dotted lines represent the fitted LOESS line (best fit).



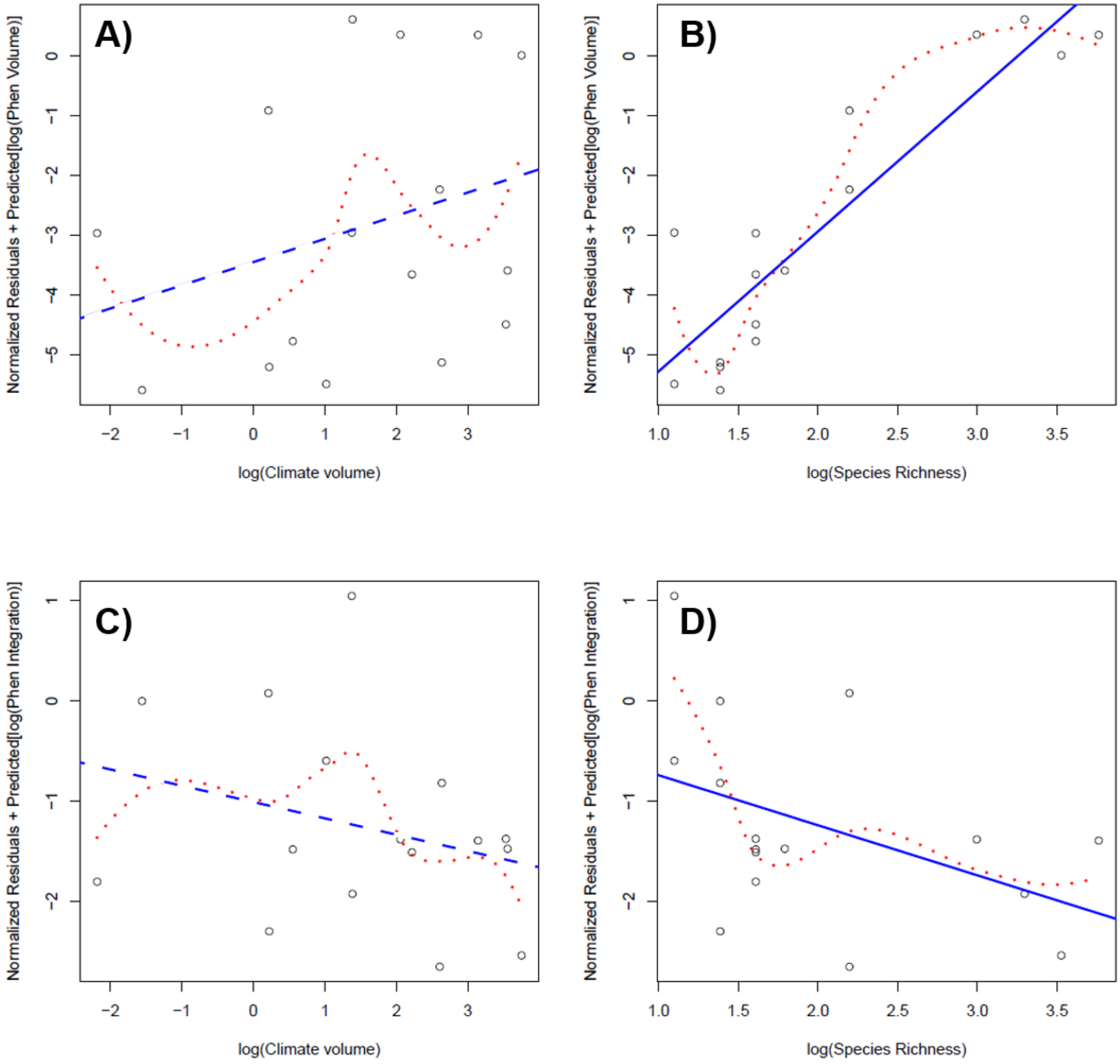
Supplementary Figure S2.9. Partial residual plots (normalized residuals + dependent variable) of the variability of minor workers' phenotypic volume (A, B) and phenotypic integration (C, D) explained by climate volume and species richness per genus while accounting for phylogenetic relationships (using PGLS). All variables were log-transformed. See Table 2.2 for the model summary. Blue solid (significant) and dashed (not significant) lines represent the partial residual line of best fit. Red dotted lines represent the fitted LOESS line (best fit).



Supplementary Figure S2.10. Partial residual plots (normalized residuals + dependent variable) of the variability of intermediate workers' phenotypic volume (A, B) and phenotypic integration (C, D) explained by climate volume and species richness per genus while accounting for phylogenetic relationships (using PGLS). All variables were log-transformed. See Table 2.2 for the model summary. Blue solid (significant) and dashed (not significant) lines represent the partial residual line of best fit. Red dotted lines represent the fitted LOESS line (best fit).



Supplementary Figure S2.11. Partial residual plots (normalized residuals + dependent variable) of the variability of major workers' phenotypic volume (A, B) and phenotypic integration (C, D) explained by climate volume and species richness per genus while accounting for phylogenetic relationships (using PGLS). All variables were log-transformed. See Table 2.2 for the model summary. Blue solid (significant) and dashed (not significant) lines represent the partial residual line of best fit. Red dotted lines represent the fitted LOESS line (best fit).



Chapter 3 Community-wide trait adaptation, but not plasticity, explain ant community structure in extreme environments

The following chapter is based on the published manuscript: Ibarra-Isassi, J., I.T. Handa, and J.P. Lessard (2023). Community-wide trait adaptation, but not plasticity, explain ant community structure in extreme environments. *Functional Ecology,* 37, 139-149.
https://doi.org/10.1111/1365-2435.14185

3.1 Abstract

Quantifying trait-environment associations can help elucidate the processes underpinning the structure of species assemblages. However, most work has focused on trait variation across rather than within species, meaning that processes operating at the intraspecific levels cannot be detected. Incorporating intraspecific trait variation in community-wide analyses can provide valuable insights about the role of morphological adaptation and plasticity on species persistence and the composition of ecological communities. Here, we assessed geographic variation in the direction (i.e., adaptation) and strength of selection, and the magnitude of plasticity, by examining community-wide trait variation in ant communities along an environmental gradient spanning 9° latitude in Quebec, Canada. Specifically, we measured 9 morphological traits related to foraging strategies, resource use and thermal regulation at 20 locations across temperate and boreal forests. We then examined how the mean and variance of these traits varied along temperature and precipitation gradients. Moreover, we examined how these trait-environment relationships varied across levels of organization, from individual workers (intraspecific) to colonies (intraspecific) and species (interspecific). We observed changes in mean trait values along environmental gradients, but very little change in variance. Specifically, we observed a decrease in the mean length of antennae and an increase in the mean eye length from mild (warm and wet) to more extreme environments (cold and dry). These shifts in trait means were mostly coordinated across organizational levels (i.e., worker, colony, and species). We also observed a general increase in trait variance from mild to extreme environments, but only at the species level. Our findings suggest that stressful environmental conditions exert a strong selection pressure on ant morphology causing shifts in optimal trait values. These adaptations may enable persistence at the northern edge of the boreal forest and therefore influence the composition of

these ant communities. Specifically, ants with large eyes and short antennae are overrepresented at the transition zone between the boreal forest and the tundra, possibly representing an adaptation to these more open habitats. Our study demonstrates that combining spatial and community-wide intraspecific functional trait data provides a promising way forward to gain new insights on trait adaptations and processes underpinning community structure along environmental gradients.

3.2 Introduction

One of the core challenges in community ecology is understanding the processes responsible for the structure and dynamics of assemblages of co-occurring organisms (Whittaker 1965, Weiher and Keddy 1999). To this aim, many studies have demonstrated the utility of traits for inferring niche-based processes operating at the species level such as environmental filtering and interspecific competition or limiting similarity (Wright et al. 2004, McGill et al. 2006, Swenson and Weiser 2010, Pollock et al. 2012, Shipley et al. 2016, Brousseau et al. 2018). These approaches assume that all individuals of a species share the same phenotype within a community and across their range while it is now well established that intraspecific trait variation can be substantial (Hulshof and Swenson 2010, Valladares et al. 2014, Classen et al. 2017, Laughlin et al. 2018b, Wieczynski et al. 2019, Brassard et al. 2020).

Community-wide patterns of individual trait variation are often used to assess the influence of niche-based processes on community structure, but rarely integrate intraspecific trait variation. Indeed, it is often assumed that knowledge of intraspecific trait variation is not necessary to infer processes shaping community structure (Siefert et al. 2015). Thus, our understanding of how intraspecific trait variation modulates species responses to environmental gradients, and, in turn, community structure and dynamics is poorly understood (Brousseau et al. 2018). Along environmental gradients, going from mild to more extreme or stressful environmental conditions, species assemblages are subject to selective pressures of variable strength and direction. Shifts in the directions of selection may lead to local adaptation and shifts in mean trait values in a community (Valladares et al. 2014). In addition, variation in the strength of selection and the magnitude of phenotypic plasticity (i.e., the ability of a genotype to produce different phenotypes as an environmental response) can affect community-level trait variance along environmental gradients (Wieczynski et al. 2019). For example, an increase in the strength of selection should decrease trait variance whereas an increase in the magnitude of plasticity should increase trait variance (Valladares et al. 2014). Therefore, considering intra- and interspecific trait variation allows inferring shifts in the direction and strength of selection, as well as plasticity, and the influence of these processes on community structure along environmental gradients.

Most functional biogeography studies use globally averaged species trait values when examining community-wide trait structure while neglecting to consider intraspecific trait variation within and between local communities (Violle et al. 2012). Evidence from studies based on global trait averages suggests that multiple dimensions of community-wide trait structure track climatic and stress gradients, with lower trait mean, variance, and smaller volume found in more variable and stressful environmental conditions (e.g., Swenson et al. 2012, Lamanna et al. 2014, de la Riva et al. 2018). These patterns are consistent with a shift in the direction of selection (measurable by community-wide mean trait value) and an increase in the strength of environmental selection (measurable by community-wide trait variance) in stressful environments (Fischer 1960, Swenson et al. 2012). To our knowledge, however, very few studies considered intraspecific variation in traits while examining community-wide shifts in mean and variance along environmental gradients (Wieczynski et al. 2019). Considering intraspecific variation within a local community could help quantify the magnitude of plasticity in response to stressful environments, which is measurable by community-wide trait variance.

Current theories differ about how inter- and intraspecific trait variation should track climate. On the one hand, current evidence based on community-wide trait variation at the interspecific level suggests low trait variation in extreme and fluctuating environments owing to strong selection or trait sorting (Šímová et al. 2018, Bruelheide et al. 2018, Wieczynski et al. 2019). On the other hand, previous research also indicates that plasticity should be higher in stressful or extreme environments, which should lead to higher trait variation at the intraspecific level in these environments (Valladares et al. 2014, Chevin and Hoffmann 2017). This discrepancy could relate to differing processes dictating trait variation at inter- vs intraspecific levels, and their influence on community structuring. Indeed, stressful or extreme environments could lower trait variation by filtering out species with suboptimal trait values, whereas those species that manage to persist could do so through plasticity, increasing intraspecific trait variation (Valladares et al. 2014, Stark et al. 2017).

Ectotherms such as ants are a near ideal study system to study trait variation along environmental gradients. In particular, the structure of their communities (i.e., composition and richness) is known to vary greatly along even very short temperature and/or precipitation gradients (Sanders et al. 2003, 2007b, Dunn et al. 2009). Moreover, ant ubiquity, ease of collection and ecological importance equally contribute to make them an excellent study system (Hölldobler and Wilson 1990, Agosti et al. 2000, Parr et al. 2017). Many studies have shown that ant community composition varies along broad environmental gradients (Lessard et al. 2007, Sanders et al. 2007b, Dunn et al. 2009, Arnan et al. 2012, Bishop et al. 2015). The replacement of species along environmental gradients is likely due to species-level trait-environment associations, but the traits mediating turnover along environmental gradients remain poorly understood (Schofield et al. 2016). Trait-environment studies in ants have focused on average species trait values and assumed intraspecific variation to be small or negligible (e.g., Arnan et al., 2014). Due to the social structure of ants, intraspecific variation can be considered between workers of the same species, regardless of the colony, and between colonies of the same species. Such intraspecific variation in ant morphology can be substantial across the range of species and correlated to large-scale abiotic gradients (Brassard et al. 2020, Oliveira et al. 2022). Furthermore, seasonal variation in temperature and precipitation are greater at northern latitudes and should promote plasticity (i.e., intraspecific trait variance). In fact, shorter growing seasons in cold environments (such as the ones found in northern latitudes) constrain ant size (Brassard et al. 2020), since their development time increases rapidly with decreasing temperatures (Penick et al. 2017). However, intraspecific variation in trait at the community-level has not been explored, and it is therefore unclear how differences in selection regime, strength of selection, or plasticity contribute to shifts in community structure along environmental gradients.

Here, we aimed to assess the relative influence of selection and plasticity as processes driving the assembly and composition of ant communities along a broad-scale environmental stress gradient. To achieve this, we examined community-wide patterns of trait mean and variance among 20 ant communities spread across a latitudinal gradient in Quebec, Canada. We tested the hypotheses that (1) a change in selection regime would select for different trait values along the latitudinal gradient resulting in a change in community-wide mean values (see Table S1 for specific trait hypotheses), (2) an increase in environmental stress at northern latitudes would lead to stronger selection pressure on traits enabling persistence in these conditions resulting in a lower community-wide trait variance, and (3) an increase in environmental unpredictability at northern latitudes would promote higher levels of plasticity resulting in a higher community-wide trait variance. We explored these patterns at the intra- and interspecific levels for a comprehensive assessment of underlying processes shaping community structure. Moreover, because ants are eusocial, meaning that an individual could be a single worker or a colony, we examined community-wide trait mean and variance across levels of organization, from individual workers (intraspecific) to colonies (intraspecific) to species (interspecific).

3.3 Methods

3.3.1 Study Sites

We measured traits on 37 ant species found in 20 sites along a latitudinal gradient in eastern Canada, specifically in the province of Quebec (Fig. 3.1). Sampling sites were in forests dominated by *Acer saccharum*, *Carya cordiformis*, *Betula alleghaniensis*, *Abies balsamea* in the Temperate zone and *Abies balsamea, Betula papyrifera*, *Picea mariana*, *P. glauca* in the Boreal zone (Baldwin et al. 2019). Sampling spanned approximately 9° in latitude (from 45° to 54° N), including an average temperature range of ~6°C (southern region) to ~-3.5°C (northern region) and an average precipitation range of ~1200 mm (southern region) to ~650 mm (northern region). This gradient was selected because it goes from low environmental stress (i.e., warm, and wet climate at low latitudes) to high environmental stress (i.e., cold, and dry climate at high latitudes). Cold and dry climates are considered extreme and/or stressful climate for ants owing to their tropical origin, which resulted in species poor and phylogenetically clustered communities in arid regions and at northern latitudes (Lessard et al. 2012). Each of the 20 sites along the latitudinal gradient were in areas away from roads, trails with high visitor traffic or any recent human disturbances (Sanders et al. 2007a). Additionally, the canopy in the selected sites was partially open, since Francoeur (2001) reported such areas to be more populated by ants, particularly in the boreal forest.

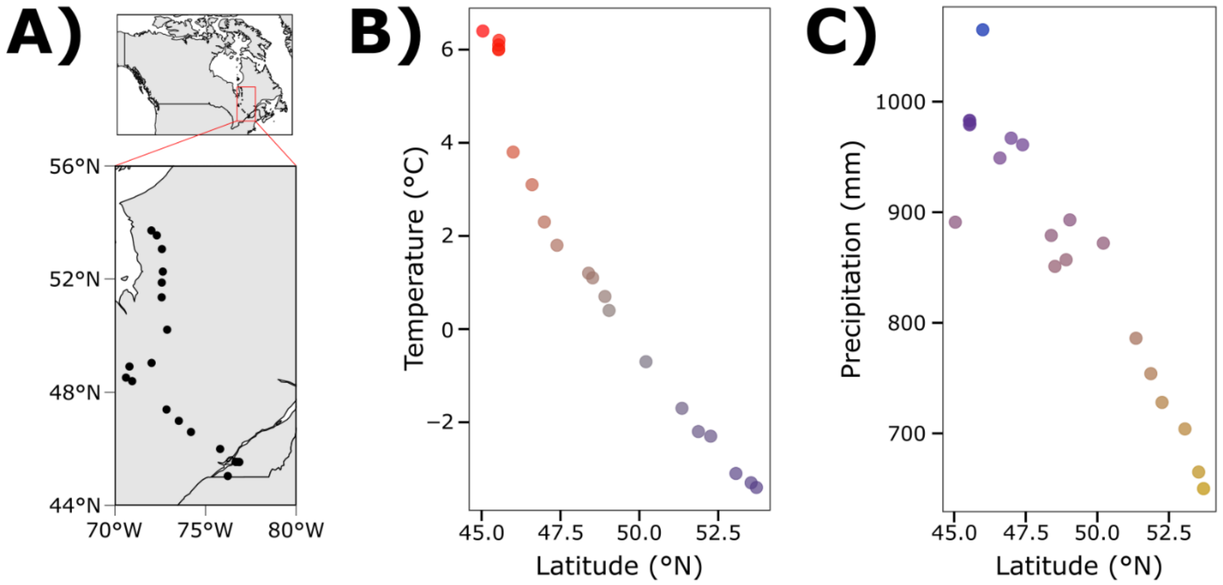


Figure 3.1. A) Distribution of sampling points across Quebec, Canada. Scatterplots of (B) temperature and (C) precipitation across the latitudinal gradient. Circles represent the 20 sampling sites where ant specimens were collected. Darker colours (red and dark blue) and lighter colours (light purple and yellow) represent higher and lower values, respectively, in temperature and precipitation.

3.3.2 Field sampling

We sampled 20 sites along the latitudinal gradient between June and August 2017. Permits were not required at most locations. Where needed due to being private property (i.e., HAVEL, VALDA, REDPI, DORVA; see Table S3.1b for list of sites), permission to sample was requested directly to the owner(s) during the day of the sampling. We standardized our site selection by choosing areas that had similar canopy cover (40-70%), similar distance to a main road and/or to a body of water (at least 100 m away). At each sampling site, we randomly established a 50×50 m plot. Plot sampling has been recommended as it can be used to map colonies which, in turn, allow to study for spatial relationships among colonies and between colonies, as well as assess environmental features (Herbers 1994, Crist and Wiens 1996). Within each corner and at the center of this plot, we placed a 10×10 m subplot. At each of these five subplots, we registered all soil and leaf-dwelling ant nests found by systematically searching in the leaf-litter, under rocks, dead trees and logs found within this area for 20 min (modified "Intensive sampling" technique described by Bestelmeyer et al., 2000). During this period, we put a flag where the colony was located and proceeded to continue our search. We only collected ant workers after this 20-min search period had ended. As collecting colonies can be labour and time intensive due to the number of workers ranging from a few tenths to a couple of thousands, we used subplots and sampling time to standardize our sampling within the 50x50 m plot (Agosti et al. 2000). Still, for every nest found, a subset of at least 5-10 workers was sampled since this has been reported to be sufficient for ant morphological trait measurements (Schofield et al. 2016, Parr et al. 2017).

3.3.3 Measuring ant functional traits

We measured nine raw morphological traits that are regularly used in the literature (Fig. 3.2; Table S3.1a). We chose these traits because they relate to locomotion, foraging, communication, environmental cue detection, and adaptation to environmental variation (Brousseau et al. 2018). Additionally, we recognize the importance of standardized protocols in trait studies (Moretti et al. 2017); thus, we followed the protocol suggested by (Parr et al. 2017).

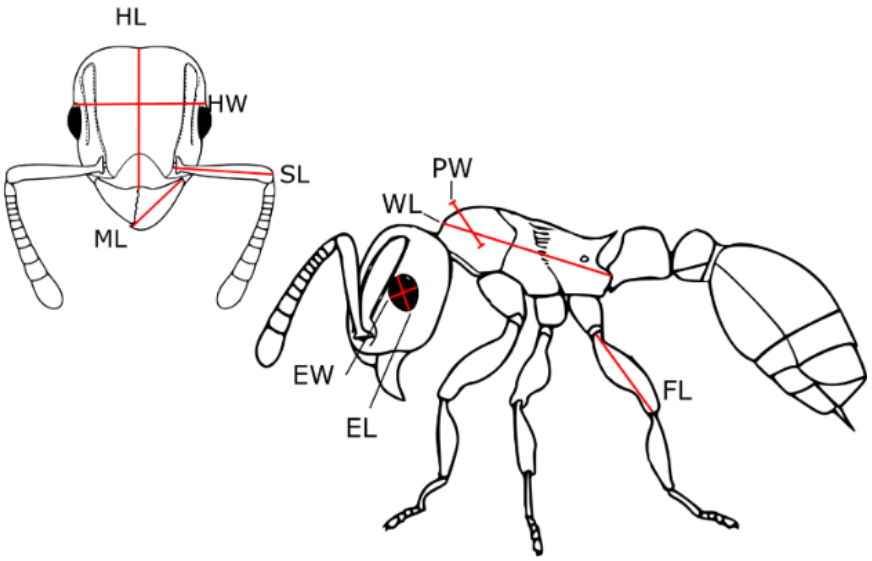


Figure 3.2. Diagram of morphometric measurements: Head Length (HL), Head Width (HW), Mandible Length (ML), Scape Length (SL), Maximum Eye Width (EW), Maximum Eye Length (EL), Pronotum Width (PW), Body Size/Weber's Length (WL), and Hind Femur Length (FL).

At each site, we found between 1 to 24 colonies per species, and we measured all nine traits on 1 to 3 individual workers from each colony and thus 3 to 72 specimens were measured per site. See Table S3.1c for number of colonies per species found at each site. Specifically, for monomorphic species, we measured 1 to 3 specimens per colony. Then, for polymorphic species (e.g., *Pheidole* spp., *Camponotus* spp.), we visually selected and measured one representative of each size class (i.e., majors, intermediate and minors). Given that polymorphic species only accounted for 8% of the total species and 6.5% of the colonies found in our study area, variation in morphology within the colony is expected to be small. Finally, for each ant, we took standard linear measurements using an ocular micrometer mounted on a dissecting microscope, accurate to 0.01 mm.

3.3.4 Environmental data

The macroenvironmental variables for each of the 20 sites: annual mean temperature (hereby 'temperature') and annual precipitation (hereby 'precipitation') were extracted from WorldClim 2.0 (Fick and Hijmans 2017). Previous studies show that temperature and precipitation are important factors influencing the composition of ant community structuring (e.g., Dunn et al. 2009, Arnan et al. 2014, Andersen et al. 2015). Additionally, both variables are limiting at northern sites, making them appropriate to include in our models.

3.3.5 Statistical analyses

3.3.5.1 *Trait variation metrics*

First, we log-transformed all trait measurements to eliminate size-dependent trait biases before analysis (Peres-Neto and Magnan 2004). Then, for each 50×50 m plot, we estimated trait mean and variance by pooling the data collected across the five subplots. We calculated the mean and variance at the worker-, colony- and species-level. At the worker-level, mean and variance was calculated using each worker individual occurring in a plot as a sample. At the colony-level, trait values were averaged across workers for each colony, regardless of species, and mean and variance were calculated using each colony as a sample. At the species-level, trait values were averaged across colonies for each species, and mean and variance were calculated using each species as a sample. At the species level, we also calculated abundance-weighted mean and variance (i.e., abundance-weighted trait moments), where abundance was the number of colonies found in a plot for a given species. Both abundance-weighted and non-weighted metrics can provide key features of functional diversity and reflect the dynamics of community structuring that may result from responses of individual organisms to environmental variables (Violle et al. 2007, Mouchet et al. 2010). Note that abundance could only be estimated at the species-level.

3.3.5.2 *Trait-environment relationships*

To assess how trait means relate to variation in temperature and precipitation, that is if there are shifts in optimal trait values along the gradient, we fit a fourth corner model using the function *traitglm* of the 'mvabund' package in R (Wang et al. 2012). This function fits a negative binomial generalized linear mixed model (GLMM) to model the occurrence (worker, colony, and species-level) or abundance (species-level only) of an organism or species *i* at site *j* (response variable) as a function of trait values and environmental variables (predictors) using the natural logarithm link function (Brown et al. 2014, ter Braak 2019). We assessed the trait-environment relationships by estimating the interactions between trait values and environmental variables and its effect on the occurrence of workers, colonies, and species (using non-weighted and abundance-weighted species trait means). This analysis essentially quantifies the influence of multiple abiotic variables on mean trait values and how this influences occurrences and provides the strength and direction of trait-environment relationships (Laughlin et al. 2018a). Environmental and trait variables were standardised by subtracting the mean of observed values from the observed value and dividing this result by standard deviation (z-standardization), to allow interpretation of coefficient sizes directly in terms of interaction strength and importance. The fourth-corner analysis generates standardized coefficient values that quantify the strength and direction of trait-environment associations. The statistical significance of this trait-environment interaction can be calculated using the *anova.traitglm* function from the 'mvabund' package, which uses row-resampling (i.e., resampling sites, but keeping species from a site together in the resample) for inference (bootstrap resamples = 100, Wang et al. 2012, Warton et al. 2015).

To assess how trait variance relates to variation in the environment, that is to evaluate the degree of phenotypic plasticity across the gradient, we first quantified the variance in trait value in each ant community following the same approach that we used to calculate the mean trait value. Then, we used linear regressions to relate trait variance to temperature and precipitation using the *lm* function in R 4.0.5 (R Core Team 2022). We used the variance of trait values as our response variable and the environmental variables as predictors.

To test that variance occurs between species rather than between individual workers or between colonies, we employed *T-statistic* metrics (Violle et al. 2012, Taudiere and Violle 2016). The *T-statistic* metrics allow the comparison of within-population variance to within-community variance and community-wide variance to regional pool trait variance.

3.4 Results

In total, across the 20 sites, we measured 1137 individuals belonging to 37 species from 4 subfamilies: Amblyoponinae, Dolichoderinae, Formicinae and Myrmicinae. The largest ants were *Camponotus herculeanus* and the smallest were *Solenopsis molesta* (WL= 4.20 mm and 0.39 mm, respectively). Excluding polymorphic species, *Aphaenogaster picea* had the largest range of body size (WL = 1.9 - 1.2 mm). Across study sites, *A. picea* also had the greatest number of colonies per site with a total of 68 colonies, and the highest number of *A. picea* colonies at a single site was 24 (MSBA-, 45.5ºN), but *A. picea* colonies were only found at the most southernmost sites (up to 47º N). They were followed by *Myrmica alaskensis* with 12 colonies at one site (LASAR, 48.9ºN), *Lasius umbratus* with 10 colonies (DPINS, 48.4ºN), and *Formica neorufibarbis* and *M. detritinodis* which had 9 colonies (MIRAB, 51.9ºN, and EASTM, 52.3ºN, respectively).

3.4.1 Trait-environment relationships across organizational levels

The fourth corner analysis revealed a range of significant interactions between mean trait values across organizational levels (Fig. 3.3; Table S3.3a). Specifically, we found that antennae length (SL) had a positive relationship with temperature across all organizational levels (Table S3.3a). Meanwhile, eye length (EL) had a negative relationship with temperature at the colony and species level, but not at the worker level (Table S3.3a). Pronotum width (PW) had a negative relationship with temperature at the individual and colony level (Table S3.3a). Other traits, such as femur (FL) and mandible (ML) length had a negative and positive relationship, respectively, at the worker level, but not at other organizational levels (Table S3.3a).

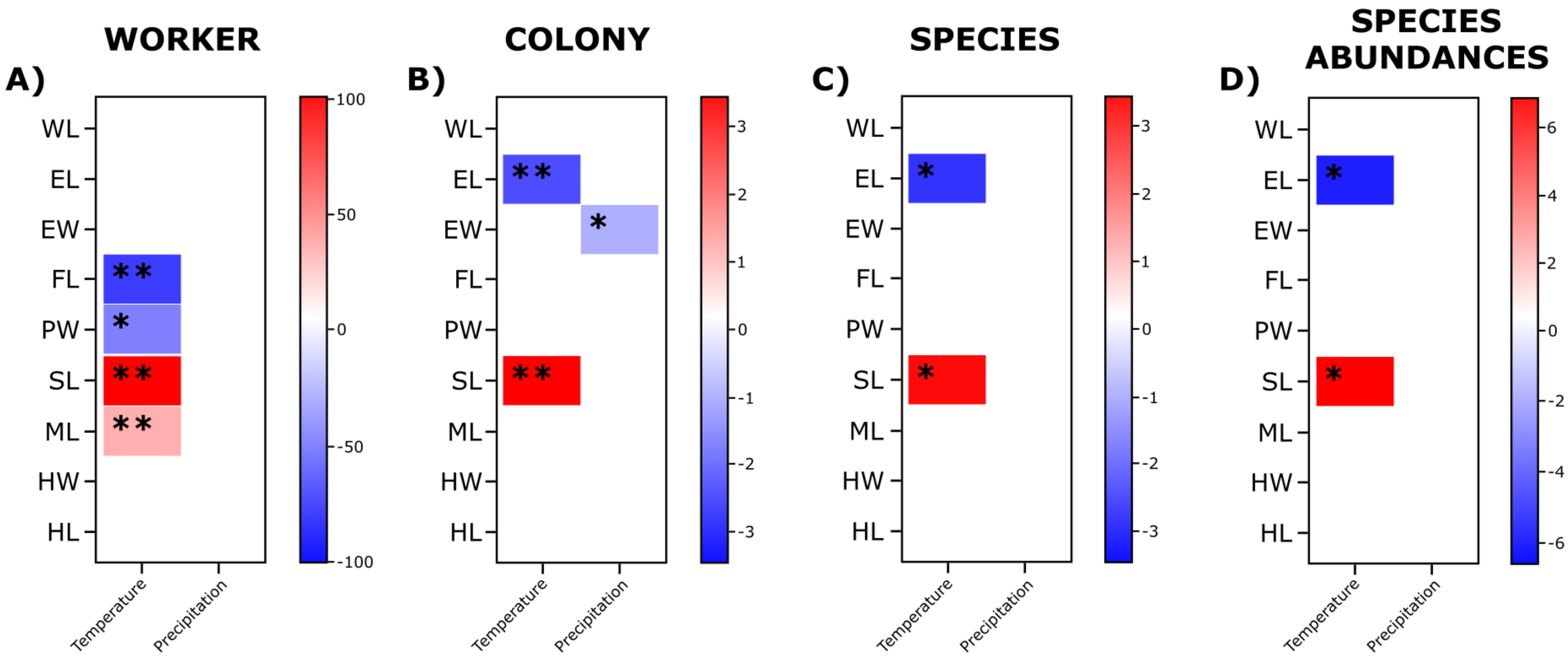


Figure 3.3. Relationships between (A) worker-, (B) colony-, (C) species- and (D) species (abundance-weighted) level trait means and environmental variables resulting from the fourth-corner analysis. Red cells and blue cells indicate positive and negative relationships, respectively. Color intensity indicates the strength of the trait-environment association. Empty cells indicate no significant relationship. Traits measured: Head Length (HL), Head Width (HW), Mandible Length (ML), Scape Length (SL), Pronotum Width (PW), Hind Femur Length (FL), Maximum Eye Width (EW), Maximum Eye Length (EL) and Body Size (WL). Environmental variables: Annual Mean Temperature and Annual Precipitation. Asterisks (*) represent significant trait-environment relationships. The number of replicates varies among sites and levels. The average number of replicates across sites was 9.2 (worker), 3.32 (colony) and 6.45 (species).

3.4.2 Trait variance across gradients

Throughout the sampled environmental gradient, we found that ants exhibit shifts in trait variance across the precipitation gradient, but not across organizational levels (Fig. 3.4; Fig. S3.1a, Table S3.3b). At the worker level, we found the variance in eye width (EW) decreased with increasing precipitation (Table S3.3b). We did not find any statistically significant trends at the colony level. Finally, at the species (abundance weighted) level we found all trait variances (except eye length -EL-) decreased with increasing mean annual precipitation values (Table S3.3b). Further, we found that most morphological variation occurs between rather than within species (Fig. S3.1b) and that the within-plot intraspecific trait variance is lower than the within-plot interspecific trait variance (*T-statistic*, Appendix S3.2).

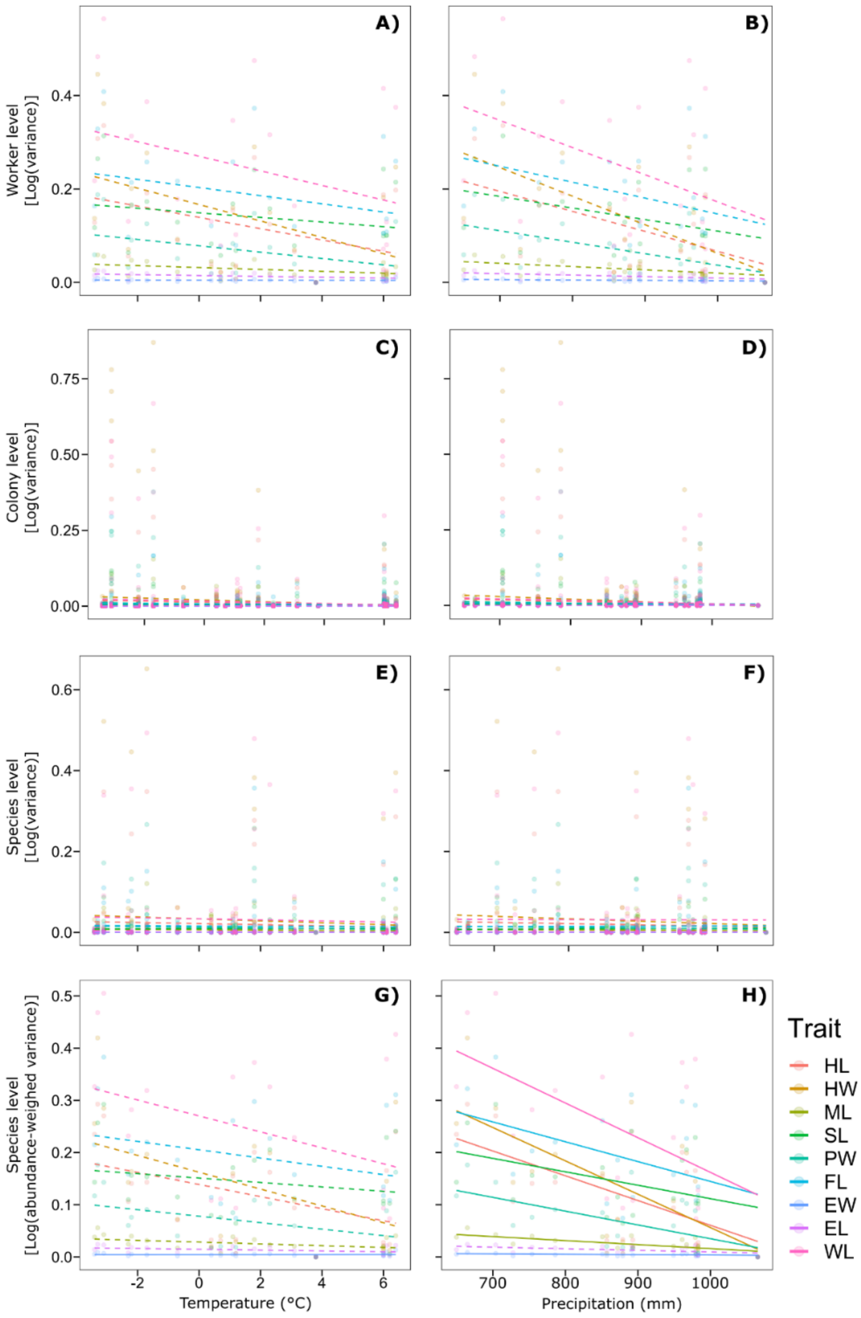


Figure 3.4. (A-B) Worker-, (C-D) colony-, (E-F) species- and (G-H) species (abundance-weighted) level trait variance across temperature (A, C, E, G) and precipitation (B, D, F, H) gradients. Solid and dashed lines represent significant and non-significant trends, respectively. Traits measured: Head Length (HL), Head Width (HW), Mandible Length (ML), Scape Length (SL), Pronotum Width (PW), Hind Femur Length (FL), Maximum Eye Width (EW), Maximum Eye Length (EL) and Body Size (WL). The number of replicates varies among sites and levels. The average number of replicates across sites was 9.2 (worker), 3.32 (colony) and 6.45 (species).

3.5 Discussion

In our study, we observed shifts in optimal (mean) trait values of ant assemblages found in extreme cold and dry environments, suggesting morphological adaptations to these climatic conditions. We hypothesized that if extreme environments exert strong selection pressures, these shifts in trait means should be accompanied by lower intra- and interspecific trait variance. Alternatively, if phenotypic plasticity enables persistence in these extreme and unpredictable environments (Chevin and Hoffmann 2017, Eriksson and Rafajlović 2022) then we expected to see higher community-wide variance. However, we found very little evidence of shifts in trait variance along environmental gradients, suggesting that neither the strength of selection nor the magnitude of plasticity is accentuated under stressful conditions (Fig. 3.4, Table S3.3b). Instead, we conclude that a shift in the selection regime causes a change in the direction of trait selection along the environmental gradient favouring morphological adaptations that enable the persistence of certain ant species at the transition zone between the boreal forest and arctic tundra.

Shifts in optimal trait values observed in our study were, for the most part, coordinated across organizational levels (Fig. 3.3). Community-wide coordinated trait shifts have been reported previously, providing evidence of widespread convergence in trait variation along climatic gradients (Hulshof and Swenson 2010, Lepš et al. 2011, Wieczynski et al. 2019). However, the differences between organizational levels in trait-environment relationships we observed suggest that the strength of the selection pressure of extreme environments is different on specific traits and at different levels (Fig. 3.3-3.4, Table S3.3a-b). For instance, we observed a relationship between temperature and pronotum width and mandible length at the worker level, but not at the colony nor species levels (Fig. 3.3, Table S3.3a). This result indicates that environmental factors such as temperature and precipitation may select certain traits of individual workers, but the pattern is not translated when integrating multiple workers at the colony or species level. Natural selection may thus be acting on two or more levels of biological hierarchy (i.e., multilevel selection) and on multiple traits in ants (Heisler and Damuth 1987, Goodnight et al. 1992, Volis et al. 2002). However, further studies are needed to provide more evidence of multilevel selection in ants between workers and colonies and between castes (e.g., reproductive vs non-reproductive).

We found evidence of community-wide adaptation for traits that influence the presence and relative abundance of species along a broad-scale environmental gradient. Notably, we found that ants with shorter antennae and larger eyes are more abundant in stressful, cold, and dry environments, and that this pattern is consistent across organizational levels. These traits have been demonstrated to be related to environmental perception and resource foraging (McLeman et al. 2002, Weiser and Kaspari 2006, Graham and Cheng 2009). Thus, our results suggest a community-wide shift from individuals and species relying on chemical cues to relying on visual cues for resource gathering and navigation. First, long winters and shorter day lengths at higher latitudes would require ants to live underground for longer and in low-light conditions, where larger eyes may be beneficial (Greiner et al. 2007, Yilmaz et al. 2014). Second, previous studies have shown that ant morphology responds predictably to habitat morphology (e.g., Weiser and Kaspari 2006, Guilherme et al. 2019, Jelley and Barden 2021). Indeed, the reduced structural complexity of ground habitats such as those found in colder environments, may favour workers with smaller antennae and longer eyes, and our study suggests that this adaptation is found across individuals, colonies, and species. However, Nooten et al. (2019) found that ants living in woody (more complex) areas have shorter antennae whereas ants living in grassy (less complex) areas had longer antennae. Though this study used an anthropogenic gradient and our study a latitudinal gradient, these contrasting results highlight the fact that other factors, such as resource availability or biogeographical history, can influence trait composition and therefore should be considered in future studies.

Though we found evidence of community-wide shift in trait means suggesting a change in selection regime, we found little evidence of changes in the strength of selection or magnitude of plasticity. Indeed, most traits (all, except eye-length) measured in this study showed an increased community-wide variance in dryer environments (i.e., northern latitudes) at the species level, but not at the worker or colony-level (intraspecific). These results suggest that phenotypic plasticity does not play a strong role in species persistence and in determining ant community structure at the northern margin of the boreal forest, which coincides with the northern range limits of all boreal ant species. Rather, extreme environments filter out individual workers and ant species with suboptimal trait values, but this filtering does not affect trait variance within sites. Together with our result that most proportion of the variance occurs between species and not within species (Fig.S3.1b), this suggests that intraspecific variance plays a minor role in community assembly at broad scales. In our study region, ant communities at northern latitudes are dominated by two lineages, Formicinae and Myrmicinae, which have been hypothesized to have traits that enable them to persist in extreme environments (Lessard et al. 2012). Our study provides further evidence of this given that most species found at our northernmost sites, *Formica* spp, *Myrmica* spp, and *Leptothorax* spp, belong to these lineages. A limitation to the above interpretation that plasticity does not structure ant communities, is that the number of specimens measured per colony was low, which could lead to an underestimation of the true variance. A larger number of within-colony replicates could provide a more robust estimation of variance and perhaps affect the variance-environment relationship (Gaudard et al. 2019). Furthermore, colony-level trait adaptation and plasticity along environmental gradients provides an interesting avenue for future research (Ibarra-Isassi et al. 2021, La Richelière et al. 2022, Gibb et al. 2023).

By analyzing trait-environment relationships across organizational levels, we can further our understanding on how individual-level trait variation influences community structuring, particularly at high latitudes (i.e., species range edges). Indeed, theory and metapopulation modelling predict an important role of phenotypic plasticity in determining species ranges and range shifts (Sultan and Spencer 2002, Hardie and Hutchings 2010). Yet, previous studies show that high levels of environmental stress select for baseline tolerances due to high plasticity costs (Van Buskirk and Steiner 2009, Hendry 2016). Our study provides evidence that shift in trait means resulting from changes in the direction of selection pressures are more important than plasticity in extreme environments often found at range edges. This finding suggests that climate change will likely cause predictable reassembly patterns with potential consequences on ecosystem functions. However, direct evidence of such reassembly patterns will require studies of trait distributions across time and the exploration of these trait shifts in response to changes in other variables such as temperature and precipitation seasonality or competition.

Ultimately, we hope our insights will help future studies aiming to bridge functional biogeography, species range shifts and community structuring. Future assemblages will represent outcomes of complex dynamics between environmental change and ecological drift. Therefore, our analysis not only strengthens our mechanistic understanding of biodiversity patterns across space, but also represents an important step towards predicting species distributions under rapidly changing environmental conditions.

3.6 Supplementary materials

APPENDIX S3.1. Supplementary information of trait measurements and sampling sites

Supplementary Table S3.1a. Description of the ant traits examined in this study and their hypothesized functional response. All measurements are in millimeters (mm).

Trait	Measurement	Hypothesized function	Hypothesized response to colder/dryer environments	Source
Antennae length (SL)	Maximum straight-line length of first antennal segment (scape)	Relates to chemical cue sensory abilities. Longer scape facilitates pheromone trail following	Decreased antennal length due to habitat simplification and decreased foraging specialization	Weiser & Kaspari 2006
Mandible length (ML)	Maximum straight-line from mandibular apex to anterior clypeal margin in full face view	Mandible size relates to predatory behaviour, with larger mandibles allowing for larger prey capture.	Decreased mandible length due to increased omnivory	Weiser & Kaspari 2006; Gibb and Cunningham 2013
Head width (HW)	Maximum horizontal length in full face view (excluding eyes)	Relates to mandible strength and predatory strategies (wider heads allow for larger mandible muscles). Narrow heads increase manoeuvrability in complex habitats or narrow gaps	Decreased head width due to decrease foraging specialists	Kaspari 1993; Sarty et al 2006
Head length (HL)	Maximum vertical length in full face view	Relates to diet, longer head length may indicate herbivory	Decreased head length due to decrease foraging specialists	Kaspari 1993; Sarty et al 2006
Eye width (EW)	Maximum horizontal diameter in lateral view	Relates to foraging behaviour and habits: nocturnal or low light level foragers have bigger eyes	Increased eye size for increased detection of optical cues in simpler habitats	Weiser and Kaspari 2006
Eye length (EL)	Maximum vertical diameter in lateral view	Relates to foraging habits and predatory behaviour.	Increased eye size for increased detection of optical cues in simpler habitats	Weiser and Kaspari 2006
Pronotum width (PW)	Pronotum longitudinal length in dorsal view	Indicative of body size and often linked with resource use and habitat manoeuvrability	Increased pronotum width (Bergmann's rule/starvation resistance hypothesis)	Wiernasz and Cole 2003; Sarty et al. 2006
Body size (WL)	Weber's length	Strongly correlates with many physiological traits	Increased body size (Bergmann's rule/starvation resistance hypothesis)	Cushman et al, 1993; Wiernasz and Cole 2003
Femur length (FL)	Hind femur length	Relates to habitat complexity adaptation: increased locomotion in simpler	Increased leg length for faster locomotion in	Kaspari and Weiser 1999; Bishop et al.

Trait	Measurement	Hypothesized function	Hypothesized response to colder/dryer environments	Source
		habitats; shorter legs increase manoeuvrability in complex habitats.	simpler habitats (size-grain hypothesis)	2016; Schofield et al. 2016

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Supplementary Table S3.1b. Environmental characteristics of each sampled site. Temperature and precipitation values were extracted from Worldclim 2.0

Site code	**Site name**	**Latitude**	**Longitude**	**Annual Mean Temperature (°C)**	**Annual Precipitation (mm)**
HAVEL	Havelock	45.04493	-73.77630	6.4	891
MSHB	Mont Saint-Hilaire (B)	45.53585	-73.14961	6.0	983
MSBA	Mont Saint-Bruno (A)	45.53974	-73.32813	6.1	979
MSHA	Mont Saint-Hilaire (A)	45.54112	-73.15727	6.0	983
MSBB	Mont Saint-Bruno (B)	4554861	-73.33466	6.2	980
VALDA	Val-David	45.99975	-74.19596	3.8	1065
REDPI	Chemin du Pin Rouge	46.59835	-75.80082	3.1	949
DOMAI	Le Domaine	46.99172	-76.46763	2.3	967
DORVA	Dorval-Lodge	47.39192	-77.14475	1.8	961
DPINS	Chemin des Pins	48.39396	-79.03118	1.2	879
DUPAR	Duparquet	48.51775	-79.37190	1.1	851
LASAR	La Sarre	48.90848	-79.18140	0.7	857
BERRY	Berry	49.03705	-77.96465	0.4	893
OUESC	Lac Ouescapis	50.20895	-77.10862	-0.7	872
RUPER	Riviere Rupert	51.35268	-77.4105	-1.7	786
MIRAB	Lac Mirabelli	51.87091	-77.40372	-2.2	754
EASTM	Eastmain	52.25950	-77.34526	-2.3	728
KM511	Bai Saint-James (KM 511)	53.05521	-77.39551	-3.1	704
LACDE	Lac Desaulniers	53.53852	-77.67651	-3.3	665
RADIS	Radisson	53.71098	-77.97479	-3.4	650

Supplementary Table S3.1c. Number of colonies per species at each sampled site. Sites are sorted from southern to northernmost latitude.

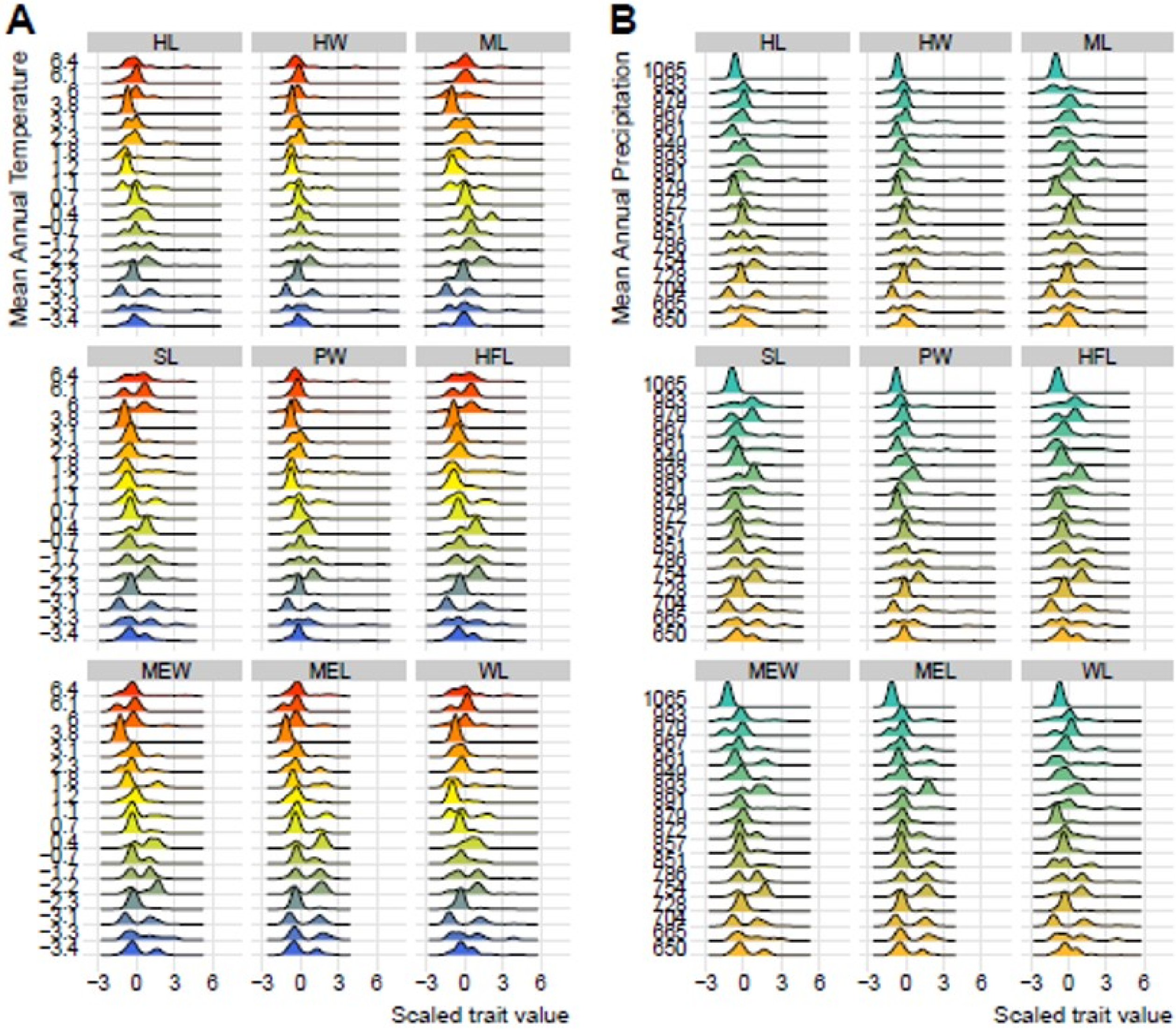
Subfamily	Species	HAVEL	MSBA-	MSHA-	MSHB-	MSBB-
Amblyoponinae	*Stigmatoma pallipes*	1	6	2	-	3
Dolichoderinae	*Dolichoderus taschenbergi*	-	-	-	-	-
	Tapinoma sessile	-	-	-	-	-
Formicinae	*Brachymyrmex depilis*	-	-	-	1	-
	Camponotus herculeanus	-	-	-	-	-
	Camponotus nearcticus	-	-	-	-	-
	Camponotus novaeboracensis	2	-	-	1	-
	Camponotus pennsylvanicus	2	-	-	3	-
	Formica aserva	-	-	-	1	-
	Formica fossaceps	-	-	-	-	-
	Formica fusca	-	-	-	-	-
	Formica glacialis	1	-	-	5	-
	Formica hewitti	-	-	-	-	-
	Formica neogagates	-	-	-	-	-
	Formica neorufibarbis	-	-	-	-	-
	Formica podzolica	-	-	-	-	-
	Formica subaenescens	-	-	-	-	-
	Formica subsericea	-	-	-	1	-
	Lasius alienus	3	-	-	5	-
	Lasius flavus	1	-	-	-	-
	Lasius nearcticus	2	2	2	-	7
	Lasius neoniger	-	-	-	-	-
	Lasius umbratus	-	-	-	1	2
Myrmicinae	*Aphaenogaster picea*	9	24	16	10	9
	Aphaenogaster rudis	-	-	-	1	-
	Leptothorax acervorum	-	-	-	-	-
	Leptothorax muscorum	-	-	-	-	-
	Leptothorax retractus	2	-	-	-	-
	Myrmecina americana	-	-	-	-	1
	Myrmica alaskensis	-	-	-	-	-
	Myrmica detritinodis	-	-	-	-	-
	Myrmica incompleta	2	-	-	1	-
	Myrmica punctiventris	-	1	2	-	-
	Solenopsis molesta	-	-	-	3	-
	Stenamma diecki	-	-	-	-	-
	Stenamma impar	-	-	-	-	1
	Temnothorax longispinosus	-	5	2	2	-

Subfamily	Species	VALDA	REDPI	DOMAI	DORVA	DPINS
Amblyoponinae	*Stigmatoma pallipes*	-	-	-	-	-
Dolichoderinae	*Dolichoderus taschenbergi*	-	-	-	-	1
	Tapinoma sessile	-	-	-	2	-
Formicinae	*Brachymyrmex depilis*	-	-	-	-	-
	Camponotus herculeanus	-	1	-	2	-
	Camponotus nearcticus	-	-	-	1	1
	Camponotus novaeboracensis	-	-	3	-	-
	Camponotus pennsylvanicus	-	-	-	-	-
	Formica aserva	-	1	-	1	-
	Formica fossaceps	-	-	-	-	-
	Formica fusca	-	-	-	-	-
	Formica glacialis	-	2	2	-	-
	Formica hewitti	-	-	-	-	-
	Formica neogagates	-	-	-	-	1
	Formica neorufibarbis	-	-	-	-	1
	Formica podzolica	-	-	-	-	-
	Formica subaenescens	-	-	-	1	-
	Formica subsericea	-	-	-	-	-
	Lasius alienus	-	-	-	-	-
	Lasius flavus	-	-	-	-	-
	Lasius nearcticus	-	-	-	-	-
	Lasius neoniger	-	4	-	-	-
	Lasius umbratus	-	-	-	-	10
Myrmicinae	*Aphaenogaster picea*	-	-	-	-	-
	Aphaenogaster rudis	-	-	-	-	-
	Leptothorax acervorum	-	-	-	-	-
	Leptothorax muscorum	-	-	1	2	4
	Leptothorax retractus	-	-	-	-	-
	Myrmecina americana	-	-	-	-	-
	Myrmica alaskensis	-	-	-	-	-
	Myrmica detritinodis	-	5	4	4	-
	Myrmica incompleta	-	7	4	-	-
	Myrmica punctiventris	-	-	-	-	-
	Solenopsis molesta	-	-	-	-	-
	Stenamma diecki	-	5	4	-	2
	Stenamma impar	4	-	-	-	-
	Temnothorax longispinosus	-	-	-	-	-

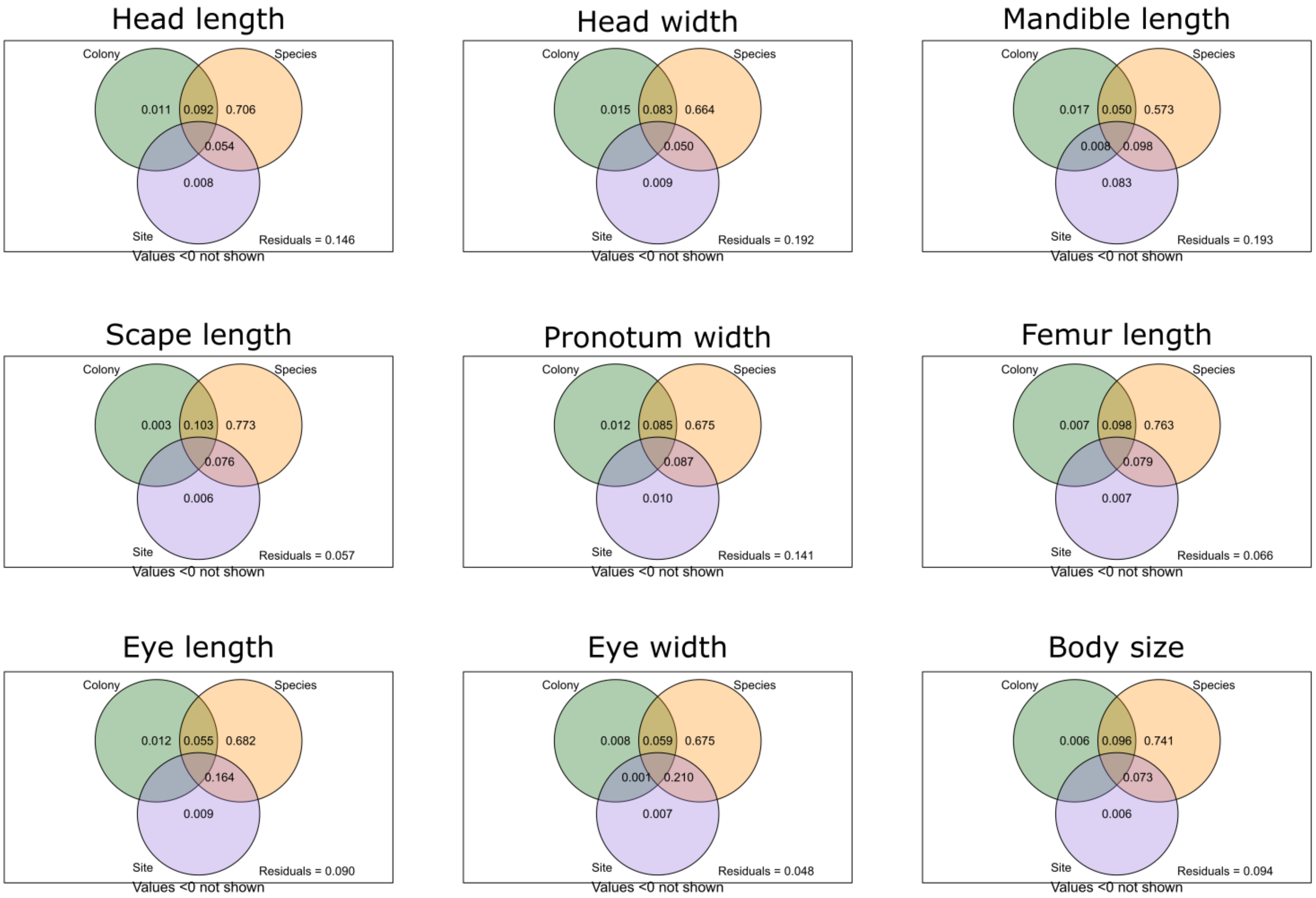
Subfamily	**Species**	**DUPAR**	**LASAR**	**BERRY**	**OUESC**	**RUPER**
Amblyoponinae	*Stigmatoma pallipes*	-	-	-	-	-
Dolichoderinae	*Dolichoderus taschenbergi*	-	-	-	-	-
	Tapinoma sessile	3	-	-	-	2
Formicinae	*Brachymyrmex depilis*	-	-	-	-	-
	Camponotus herculeanus	-	-	-	1	2
	Camponotus nearcticus	-	-	-	-	-
	Camponotus novaeboracensis	-	-	-	-	-
	Camponotus pennsylvanicus	-	-	-	-	-
	Formica aserva	4	1	-	-	-
	Formica fossaceps	-	-	-	1	-
	Formica fusca	1	-	-	-	-
	Formica glacialis	-	-	-	-	-
	Formica hewitti	-	-	1	-	2
	Formica neogagates	-	-	-	-	6
	Formica neorufibarbis	-	3	8	2	2
	Formica podzolica	-	-	1	-	-
	Formica subaenescens	-	1	-	-	-
	Formica subsericea	-	-	-	-	-
	Lasius alienus	1	-	-	-	-
	Lasius flavus	-	-	-	-	-
	Lasius nearcticus	-	-	-	-	-
	Lasius neoniger	-	-	-	-	-
	Lasius umbratus	-	-	-	-	-
Myrmicinae	*Aphaenogaster picea*	-	-	-	-	-
	Aphaenogaster rudis	-	-	-	-	-
	Leptothorax acervorum	-	-	-	-	-
	Leptothorax muscorum	3	1	1	2	1
	Leptothorax retractus	-	-	-	-	-
	Myrmecina americana	-	-	-	-	-
	Myrmica alaskensis	-	12	-	6	6
	Myrmica detritinodis	2	1	4	-	-
	Myrmica incompleta	4	3	-	-	-
	Myrmica punctiventris	-	-	-	-	-
	Solenopsis molesta	-	-	-	-	-
	Stenamma diecki	-	-	-	-	1
	Stenamma impar	-	-	-	-	-
	Temnothorax longispinosus	-	-	-	-	-

Subfamily	**Species**	**MIRAB**	**EASTM**	**KM511**	**LACDE**	**RADIS**
Amblyoponinae	*Stigmatoma pallipes*	-	-	-	-	-
Dolichoderinae	*Dolichoderus taschenbergi*	-	-	-	-	-
	Tapinoma sessile	2	-	2	-	-
Formicinae	*Brachymyrmex depilis*	-	-	-	-	-
	Camponotus herculeanus	1	1	4	1	1
	Camponotus nearcticus	-	-	-	-	-
	Camponotus novaeboracensis	-	-	-	-	-
	Camponotus pennsylvanicus	-	-	-	-	-
	Formica aserva	1	2	1	1	1
	Formica fossaceps	-	-	-	-	-
	Formica fusca	-	-	-	-	-
	Formica glacialis	-	-	-	-	-
	Formica hewitti	-	-	-	-	-
	Formica neogagates	-	-	-	-	-
	Formica neorufibarbis	9	1	6	3	2
	Formica podzolica	-	-	-	-	4
	Formica subaenescens	-	-	-	-	-
	Formica subsericea	-	-	-	-	-
	Lasius alienus	-	-	-	-	-
	Lasius flavus	-	-	-	-	-
	Lasius nearcticus	-	-	-	-	-
	Lasius neoniger	-	-	-	-	-
	Lasius umbratus	-	-	-	-	-
Myrmicinae	*Aphaenogaster picea*	-	-	-	-	-
	Aphaenogaster rudis	-	-	-	-	-
	Leptothorax acervorum	-	-	2	-	-
	Leptothorax muscorum	1	3	7	4	3
	Leptothorax retractus	-	-	-	-	-
	Myrmecina americana	-	-	-	-	-
	Myrmica alaskensis	-	9	-	2	6
	Myrmica detritinodis	3	9	-	2	5
	Myrmica incompleta	-	-	-	-	-
	Myrmica punctiventris	-	-	-	-	-
	Solenopsis molesta	-	-	-	-	-
	Stenamma diecki	-	-	-	-	-
	Stenamma impar	-	-	-	-	-
	Temnothorax longispinosus	-	-	-	-	-

Supplementary Figure S3.1a. Trait frequency distributions along temperature (A) and precipitation (B) gradients for workers. Trait values have been scaled for visualization. Traits measured: Head Length (HL), Head Width (HW), Mandible Length (ML), Scape Length (SL), Pronotum Width (PW), Hind Femur Length (HFL), Maximum Eye Width (MEW), Maximum Eye Length (MEL) and Body Size (WL).



Supplementary Figure S3.1b. Proportion of specific trait variation explained by colony, species, and site variables. On average, colony explained 1%, species 69.5%, and site 1.6% of trait variation.



APPENDIX S3.2. T-statistics analyses

Methods

We computed three observed trait variance ratios (TIP/IC, TIC/IR and TPC/PR) across our sampled gradient (Violle et al. 2012, Taudiere and Violle 2016). The three ratios were calculated as follows:

1. TIP/IC, the ratio of within-population variance (individual within population) to total within-community variance (individual within community). This ratio measures the strength of the internal filtering affecting individuals and reflects niche packing among the species of the community. Higher the values of TIP/IC can be interpreted as higher niche overlap among coexisting species.
2. TIC/IR, the ratio of community-wide variance (individual within community) to total variance in the regional pool (individual within region). This ratio allows the assessment of external-to-community filtering strength when accounting for individual differences (i.e., intraspecific variation). The higher the value of TIC/IR, the higher overlap of community trait distributions after accounting for intraspecific variation.
3. TPC/PR, the ratio of community-wide variance to total variance in the regional pool. This ratio allows the assessment of external-to-community filtering strength without accounting for individual differences (i.e., no intraspecific variation). The higher the value of TPC/PR, the higher overlap of community trait distributions at the species level.

We compared observed ratios with null models, three null models were created as following: 1) TIP/IC: randomization of individual trait values within the community, with the null hypothesis being that there is no internal filtering (i.e., trait values independent of species identity); 2) TIC/IR: randomized without replacement of individual trait values belonging to all plots, with the null hypothesis there is no external filtering (i.e., individual trait values are randomly selected from regional pool); 3) TPC/PR: assigned a plot-level value to each individual and drawn without replacement of plot level trait values belonging to all plots, with the null hypothesis that there is no species-based external filtering (i.e., mean trait values for a species is randomly selected from regional pool). All these null models were run 999 times. We then calculated the standardized effect sizes (SES) as: SES = (Tobs−Tnull)/sdTnull, where Tobs is the observed ratio value, Tnull is the mean of the ratios generated by the null models, and sdTnull is the standard deviation of these null ratios.

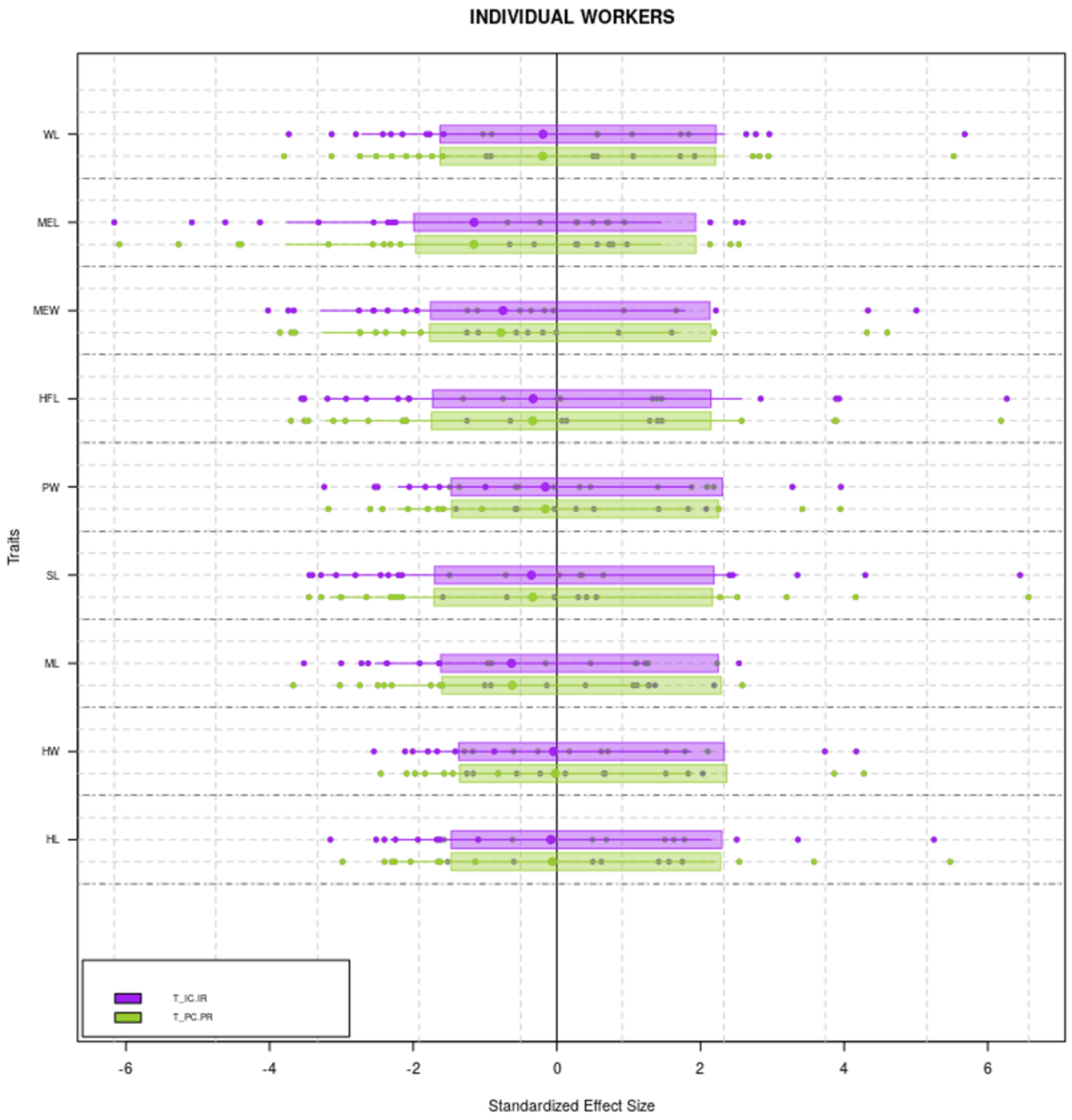
Results

The TIP/IC metric, which compares within-community intraspecific to interspecific variation, was significantly lower than null expectations for all nine traits (Fig. S3.2a-c). The individual (TIC/IR) and species (TPC/PR) level trait variance ratios that compare community patterns to the regional species pool were not significantly different from null expectations based on randomizations.

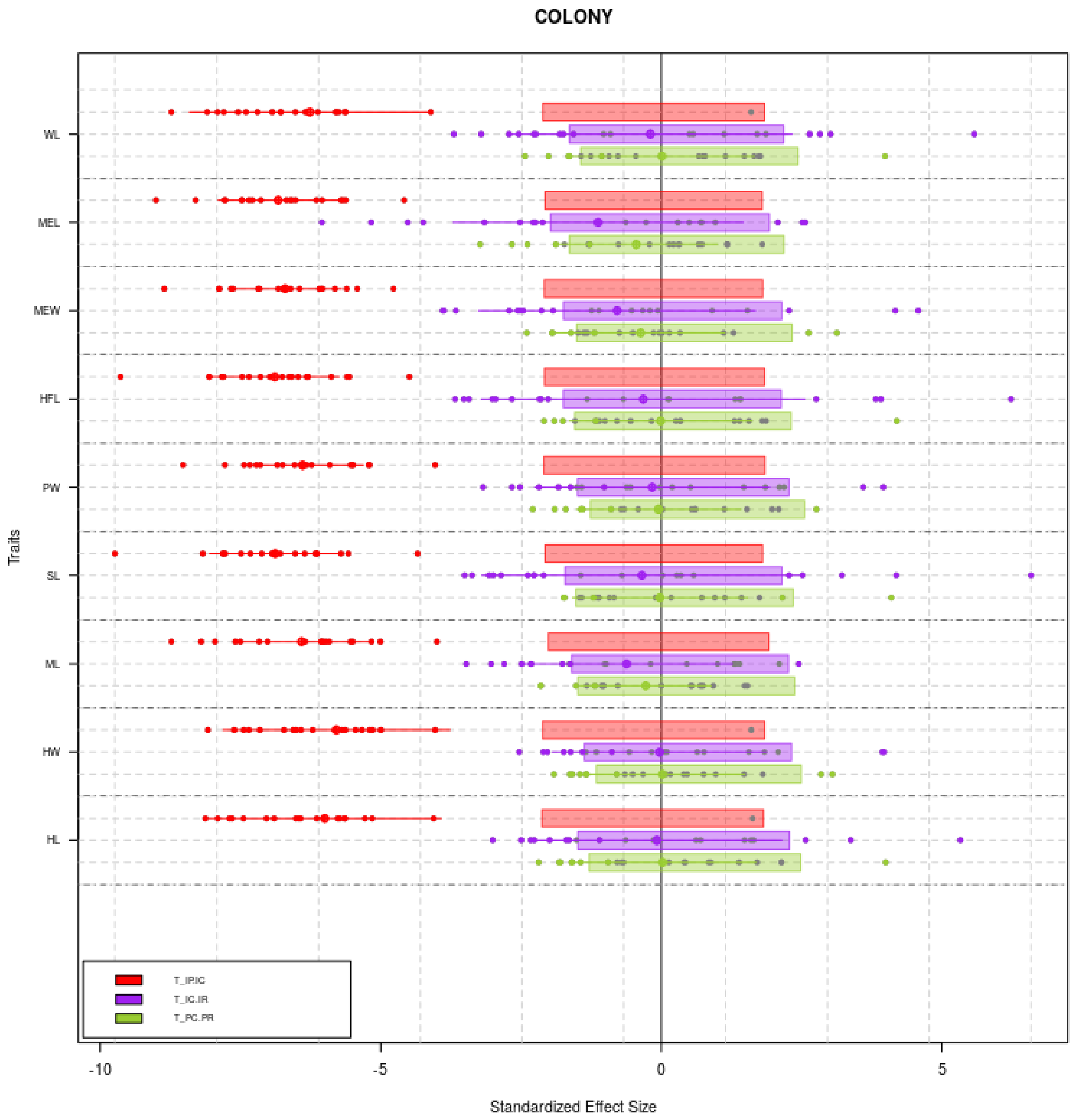
References

Taudiere, A., & Violle, C. (2016). cati: an R package using functional traits to detect and quantify multi-level community assembly processes. Ecography, 39(7), 699–708. https://doi.org/10.1111/ecog.01433

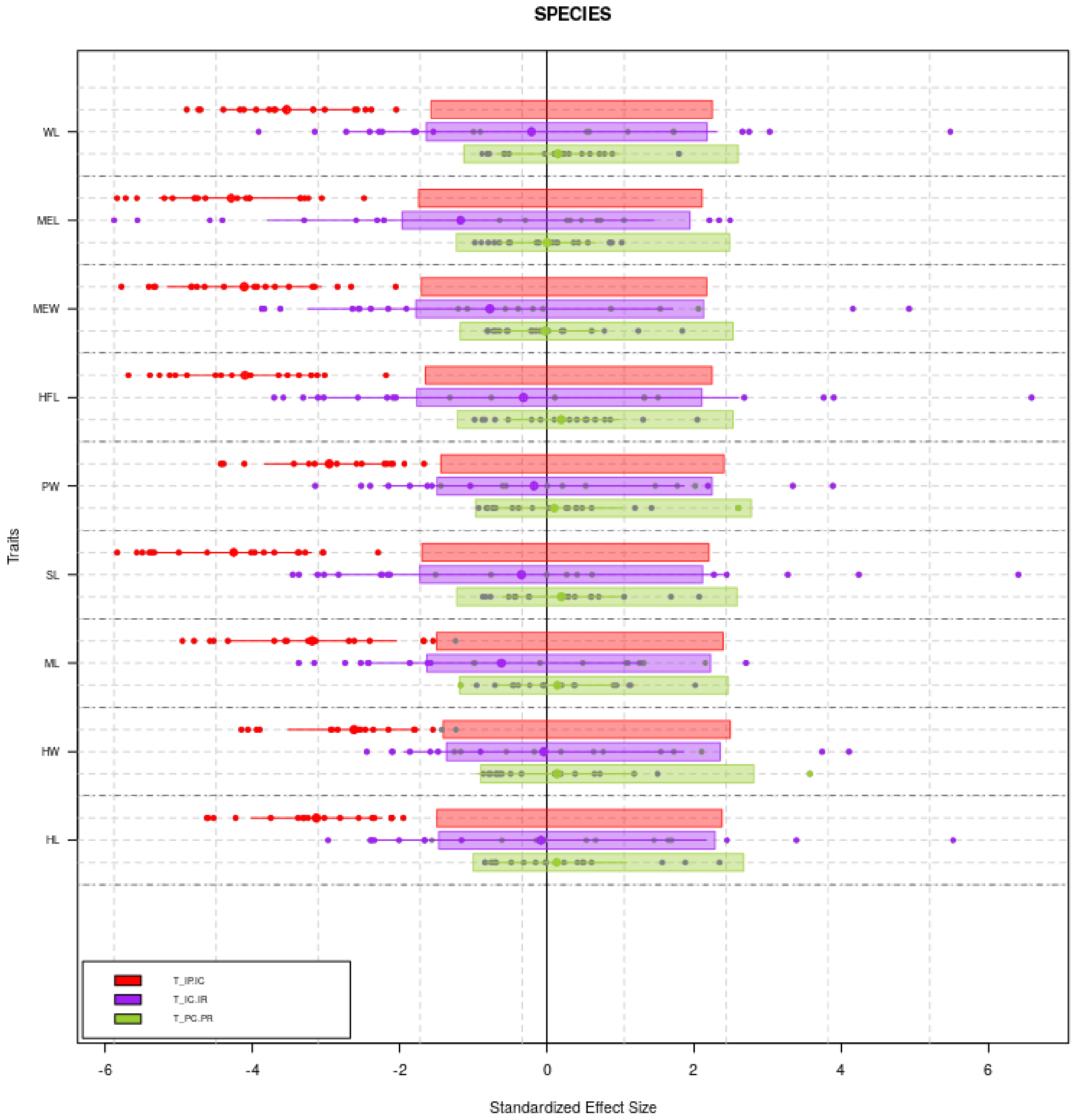
Violle, C., Enquist, B. J., McGill, B. J., Jiang, L., Albert, C. H., Hulshof, C., Jung, V., & Messier, J. (2012). The return of the variance: intraspecific variability in community ecology. Trends in Ecology & Evolution, 27(4), 244–252. https://doi.org/10.1016/j.tree.2011.11.014



Supplementary Figure S3.2a. Standardized effect sizes (SES) of T-statistics for the 9 measured traits analyzed at the individual worker level. The boxes indicate the confidence interval of the null model for each trait variance ratio. Each colored dot represents the SES value of one community when it is deviated from the null model. The larger, crossed circles and segments respectively represent the mean and the standard deviation of the SES values for a given trait. For a given trait variance ratio, the mean of the SES (larger crossed circle) is significantly different from the null distribution if not embedded within the colored box.



Supplementary Figure S3.2b. Standardized effect sizes (SES) of T-statistics for the 9 measured traits analyzed at the colony level. The boxes indicate the confidence interval of the null model for each trait variance ratio. Each colored dot represents the SES value of one community when it is deviated from the null model. The larger, crossed circles and segments respectively represent the mean and the standard deviation of the SES values for a given trait. For a given trait variance ratio, the mean of the SES (larger crossed circle) is significantly different from the null distribution if not embedded within the colored box.



Supplementary Figure S3.2c. Standardized effect sizes (SES) of T-statistics for the 9 measured traits analyzed at the species level. The boxes indicate the confidence interval of the null model for each trait variance ratio. Each colored dot represents the SES value of one community when it is deviated from the null model. The larger, crossed circles and segments respectively represent the mean and the standard deviation of the SES values for a given trait. For a given trait variance ratio, the mean of the SES (larger crossed circle) is significantly different from the null distribution if not embedded within the colored box.

APPENDIX S3.3. Fourth corner and Generalized Linear Mixed Model analyses coefficients.

Supplementary Table S3.3a. Fourth-corner analysis results across organizational levels (i.e., worker, colony, and species) trait means and environmental variables. Significant values are highlighted in bold.

Organizational level	Trait	Mean Annual Temperature		Mean Annual Precipitation	
		χ^2	p	χ^2	p
Worker	Head Length (HL)	0.282	0.618	0.856	0.250749
	Head Width (HW)	0.796	0.207	1.03	0.148851
	Mandible Length (ML)	**2.456**	**0.001**	0.314	0.598402
	Scape Length (SL)	**2.85**	**0.001**	0.257	0.677323
	Pronotum Width (PW)	**2.092**	**0.010**	0.988	0.168831
	Femur Length (FL)	**2.196**	**0.003**	0.637	0.368631
	Eye Width (EW)	0.962	0.152	1.452	0.081918
	Eye Length (EL)	0.482	0.409	1.104	0.178821
	Weber's Length (WL)	0.162	0.798	0.376	0.61039
Colony	Head Length (HL)	0.801	0.366	1.168	0.257
	Head Width (HW)	1.403	0.149	0.339	0.723
	Mandible Length (ML)	1.306	0.500	1.313	0.208
	Scape Length (SL)	**4.789**	**0.009**	0.219	0.891
	Pronotum Width (PW)	1.556	0.139	0.042	0.961
	Femur Length (FL)	1.205	0.198	1.475	0.149
	Eye Width (EW)	1.549	0.198	**2.291**	**0.049**
	Eye Length (EL)	**4.683**	**0.009**	1.484	0.188
	Weber's Length (WL)	0.821	0.376	0.492	0.644
Species	Head Length (HL)	0.072	0.970	1.536	0.1584
	Head Width (HW)	0.039	0.941	0.27	0.8218
	Mandible Length (ML)	0.285	0.802	0.488	0.6139
	Scape Length (SL)	**2.244**	**0.030**	0.445	0.6634
	Pronotum Width (PW)	0.318	0.772	0.833	0.4653
	Femur Length (FL)	0.676	0.416	1.14	0.3069
	Eye Width (EW)	1.167	0.287	1.028	0.3861
	Eye Length (EL)	**2.811**	**0.010**	0.701	0.495
	Weber's Length (WL)	0.35	0.683	0.617	0.5743
Species (abundance-weighted)	Head Length (HL)	0.504	0.693	1.791	0.1584
	Head Width (HW)	0.516	0.693	0.565	0.5941
	Mandible Length (ML)	0.019	0.990	0.871	0.3861
	Scape Length (SL)	**2.89**	**0.030**	0.664	0.6139
	Pronotum Width (PW)	1.359	0.248	1.364	0.2574
	Femur Length (FL)	0.88	0.465	1.116	0.3663
	Eye Width (EW)	2.055	0.109	2.233	0.0891

Organizational level	Trait	Mean Annual Temperature		Mean Annual Precipitation	
		χ^2	*p*	χ^2	*p*
	Eye Length (EL)	**3.932**	**0.010**	1.876	0.198
	Weber's Length (WL)	0.443	0.733	0.759	0.5347

Supplementary Table S3.3b. Linear regression estimates of variance across organizational levels (i.e., worker, colony, and species) and environmental variables. Significant values are highlighted in bold.

Organizational level	Trait	R^2	Mean Annual Temperature			Mean Annual Precipitation		
			Slope	*t*	*p*	*Slope*	*t*	*p*
Worker	Head Length (HL)	0.25	0.08	0.212	0.83	-0.58	-1.373	0.19
	Head Width (HW)	0.31	0.08	0.205	0.84	-0.63	-1.556	0.14
	Mandible Length (ML)	0.22	0.06	0.151	0.88	-0.53	-1.228	0.24
	Scape Length (SL)	0.22	0.41	0.926	0.37	-0.72	-1.622	0.12
	Pronotum Width (PW)	0.24	0.13	0.301	0.77	-0.61	-1.408	0.18
	Femur Length (FL)	0.14	0.15	0.339	0.74	-0.49	-1.091	0.29
	Eye Width (EW)	0.25	0.81	1.916	0.07	**-1.01**	**-2.355**	**0.03**
	Eye Length (EL)	0.24	0.04	0.103	0.92	-0.52	-1.221	0.24
	Weber's Length (WL)	0.17	0.13	0.298	0.77	-0.53	-1.193	0.25
Colony	Head Length (HL)	0.02	-0.12	-1.023	0.31	-0.02	-0.146	0.88
	Head Width (HW)	0.02	-0.13	-1.096	0.27	-0.01	-0.084	0.93
	Mandible Length (ML)	0.02	-0.18	-1.545	0.123	0.05	0.468	0.64
	Scape Length (SL)	0.01	0.07	0.629	0.53	-0.12	-1.065	0.29
	Pronotum Width (PW)	0.02	-0.13	-1.101	0.27	-0.01	-0.028	0.97
	Femur Length (FL)	0.01	-0.04	-0.323	0.75	-0.01	-0.048	0.96
	Eye Width (EW)	0.01	0.03	0.218	0.83	-0.07	-0.629	0.53
	Eye Length (EL)	0.01	-0.1	-0.856	0.39	0.02	0.164	0.87
	Weber's Length (WL)	0.01	-0.13	-1.098	0.27	0.01	0.044	0.97
Species	Head Length (HL)	0.04	-0.12	-0.449	0.66	-0.07	-0.3	0.76
	Head Width (HW)	0.04	-0.18	-0.702	0.49	-0.02	-0.062	0.95
	Mandible Length (ML)	0.04	-0.23	-0.876	0.38	0.04	0.15	0.88
	Scape Length (SL)	0.03	0.25	0.975	0.33	-0.33	-1.344	0.18
	Pronotum Width (PW)	0.04	-0.19	-0.728	0.47	-0.01	-0.017	0.98
	Femur Length (FL)	0.03	-0.06	-0.236	0.81	-0.1	-0.406	0.69
	Eye Width (EW)	0.03	-0.1	-0.39	0.69	-0.08	-0.341	0.73
	Eye Length (EL)	0.05	-0.15	-0.589	0.56	-0.06	-0.237	0.81
	Weber's Length (WL)	0.04	-0.19	-0.745	0.46	0.01	0.004	0.99
	Head Length (HL)	0.43	0.04	1.117	0.28	**-0.1**	**-2.641**	**0.02**
	Head Width (HW)	0.46	0.05	1.055	0.31	**-0.13**	**-2.731**	**0.01**

Organizational level	**Trait**	**R^2**	**Mean Annual Temperature**			**Mean Annual Precipitation**		
			Slope	*t*	*p*	*Slope*	*t*	*p*
Species (abundance-weighted)	Mandible Length (ML)	0.47	0.01	1.603	0.13	**-0.02**	**-3.133**	**0.01**
	Scape Length (SL)	0.28	0.06	1.603	0.13	**-0.09**	**-2.398**	**0.03**
	Pronotum Width (PW)	0.38	0.03	1.186	0.25	**-0.06**	**-2.525**	**0.02**
	Femur Length (FL)	0.27	0.06	1.171	0.26	**-0.11**	**-2.123**	**0.04**
	Eye Width (EW)	**0.35**	**0.01**	**2.68**	**0.02**	**-0.01**	**-3.004**	**0.01**
	Eye Length (EL)	0.26	0.01	0.622	0.54	-0.01	-1.711	0.11
	Weber's Length (WL)	0.34	0.09	1.127	0.28	**-0.18**	**-2.325**	**0.03**

Chapter 4 Shade-growing practices lessen the impact of coffee plantations on multiple dimensions of ant diversity

The following chapter is based on the published manuscript: Ibarra-Isassi, J, Handa, IT, Arenas-Clavijo, A, Escobar-Ramírez, S, Armbrecht, I, Lessard, J-P. (2021) Shade-growing practices lessen the impact of coffee plantations on multiple dimensions of ant diversity. *Journal Applied Ecology,* 58, 919– 930. https://doi.org/10.1111/1365-2664.13842

4.1 Abstract

Land use management influence changes in biodiversity beyond the targeted species. Management practices in coffee plantations have shifted from coffee growing below accompanying (shade) trees, to intensified monocultures in which coffee grows fully exposed to the sun. Anthropogenic disturbance causes changes in species composition relative to adjacent natural patches and reduces their biotic heterogeneity. Here, we assessed the impact of coffee plantation management practices on the taxonomical, phylogenetic, and functional composition of ant communities, an ecologically dominant group and crucial biological pest controller in these agroecosystems. We hypothesized that shade-grown coffee plantations would harbor ant communities similar to those of nearby forest patches, but dissimilar to those of intensified monocultures. We surveyed ant diversity in eight shade-grown coffee farms, eight intensive coffee monocultures and eight forest patches. We used a combination of active and passive sampling methods over two field campaigns spanning six months. Our results support our hypothesis for all diversity dimensions. Additionally, ant communities in intensified monocultures were taxonomically and functionally, but not phylogenetically, more homogeneous than those found in forest patches and shade-grown plantations.

Synthesis and applications. Our findings support the idea that practices in shade-grown coffee plantations buffer the impoverishment of multiple diversity dimensions after forest conversion. Additionally, we identified that leaf-litter depth and number of twigs mitigate ant diversity loss which, in turn, can favour the presence of potential biocontrol agents. By assessing and integrating multiple biodiversity dimensions into management strategies, farmers and interested parties can minimize future biodiversity and ecosystem service loss.

4.2 Introduction

The conversion of natural forests to croplands degrades natural ecosystems and increases the rate of biodiversity loss, increasingly threatening biodiversity worldwide (Newbold et al. 2015, Beckmann et al. 2019). Pervasive land conversion results from the increasing demand for food production that is causing farmers to transition from traditional, sustainable practices to more intensive ones (Rudel et al. 2009). These habitat shifts cause biodiversity decline mainly through the loss of forest cover (Koh et al. 2004), especially in tropical regions (Vergara and Badano 2009). Coffee production, which is concentrated in the most biodiverse regions of the world, is an important cause of natural habitat loss. Between 1994 and 2017, ~10 million hectares/year of tropical forests were transformed to coffee crops (FAO 2019). Nevertheless, the choice of management practices can affect biodiversity and associated ecosystem services.

Coffee producers use various management strategies for its cultivation. Farmers traditionally grow coffee in the understory of indigenous shade trees, providing refuges for accompanying biodiversity (Perfecto et al. 1996, Moguel and Toledo 1999). This management practice (hereby shade-grown) increases the sustainability of landscape and livelihoods of farmers (Toledo and Moguel 2012). However, many farmers transition to unshaded monocultures (hereby sun-grown) to improve the yield (Rappole et al. 2003). The removal of accompanying vegetation increases erosion, chemical runoff, and consolidation, combined with a lower long-term sustainability of the ecosystem (Perfecto et al. 1996, Rappole et al. 2003). Moreover, the lack of accompanying vegetation changes the resources (e.g., nesting sites, prey) available for other taxa, causing a decline in species richness when compared to shaded systems (Philpott et al. 2008). This decline could translate to changes in the community structure of keystone taxa, altering ecological processes that regulate ecosystem functions (Naeem et al. 1994, Symstad et al. 1998). Thus, studies focusing on the changes of keystone taxa become essential for predicting the influence of management approaches on ecosystem services provided by local biodiversity (Power et al. 1996).

In coffee agroecosystems, such keystone species include ants, which are abundant and important biological control agents (Philpott and Armbrecht 2006, Morris et al. 2018). Agricultural systems harbor low species richness of ants, particularly of twig-nesting and litter-dwelling ants (Perfecto and Vandermeer 2002, Armbrecht and Perfecto 2003, De la Mora et al.

2013). Moreover, management strategies influence ant behaviour and their potential for providing ecosystem services, such as pest control (Armbrecht and Gallego 2007, Jiménez-Soto et al. 2019, Aristizábal and Metzger 2019). Ant diversity loss in coffee plantations may exacerbate declines in ecosystem functioning due to already low levels of functional redundancy in these systems (Bihn et al. 2010). However, studies rarely address ant functional diversity in agroecosystems and could be a better proxy for ecosystem service loss.

Land conversion not only alters the composition of communities, but also the level of redundancy (Olden et al. 2004, Rodrigues et al. 2013). Biotic homogenization is the increase in the similarity of two or more species assemblages caused by the extinction or introduction of species (McKinney and Lockwood 1999). Although the causes and consequences of taxonomic homogenization have received much attention, fewer studies have focused on either the functional or phylogenetic dimensions of this process (Olden et al. 2018). Novel selective pressures deriving from land conversion and agricultural intensification may act differentially at the taxonomic, phylogenetic, and functional levels (Olden 2006, Devictor et al. 2008), and have different impact on ecosystem function, thus making integrative approaches crucial for biodiversity assessments. Both functional and phylogenetic homogenization occur when species invasions and extinctions are related to intrinsic life-history traits of closely related species (Devictor et al. 2008, Blackburn and Jeschke 2009). However, the degree of homogenization will depend on the type and intensity of disturbance, the ecology of the organism, and the surrounding landscape matrix (Sonnier et al. 2014, Villéger et al. 2014, Gámez-Virués et al. 2015, Rousseau et al. 2019). The increase in phylogenetic and functional similarity may lead to less stable, less productive, and extinction-prone ecosystems which can translate to a decrease in the provision of ecosystem services (van der Plas et al. 2016).

In this study, we assessed the effects of two different coffee plantation management practices on the taxonomic, phylogenetic, and functional diversity of ant communities. We asked whether shade-grown coffee practices could buffer the effect of land conversion on ant community structure. Specifically, we tested the hypotheses that ant communities in shade-grown coffee plantations (1) do not differ significantly in composition from forest patches but differ significantly from sun-grown coffee plantations; and (2) are as heterogeneous as in forest patches, but less homogeneous than in sun-grown coffee plantations. In addition, we examined which environmental variables underly changes in ant communities within and between habitats.

4.3 Methods

4.3.1 Study Area

We conducted our study in Cauca, Colombia, between 2°49’44” - 2°51’32”N and 76°34’8’ - 76°33’25”W; with an altitude range of 1336-1538 m, with mean annual temperature and precipitation of 21.5°C and 2191 mm, respectively. This region has two precipitation peaks during the year April-May and October-November (IDEAM 2019). This is an area dominated primarily by shade-grown coffee (commercial polycultures, and shaded monocultures *sensu* Moguel and Toledo 1999). We selected eight shade-grown and eight sun-grown coffee plantations with areas between 0.5 and 8 ha, and where owners reported a low number of agrochemicals used and similar management practices (see Appendix S4.2: Table S4.2a in supporting information). In shade-grown coffee plantations, farmers planted shade trees (*Inga spectabilis* and/or *I. edulis,* Fabaceae) interspersed with rows of coffee bushes. In sun-grown coffee plantations, farmers planted *Musa paradisiaca* (Musaceae), *Manihot esculenta* (Euphorbiaceae) or *Citrus sinensis* (Rutaceae) on the edge of the plots, providing no shade to coffee bushes. To establish a comparison with residual forests, we collected samples from eight neighbouring forest patches no bigger than 2 ha (Fig. 4.1).

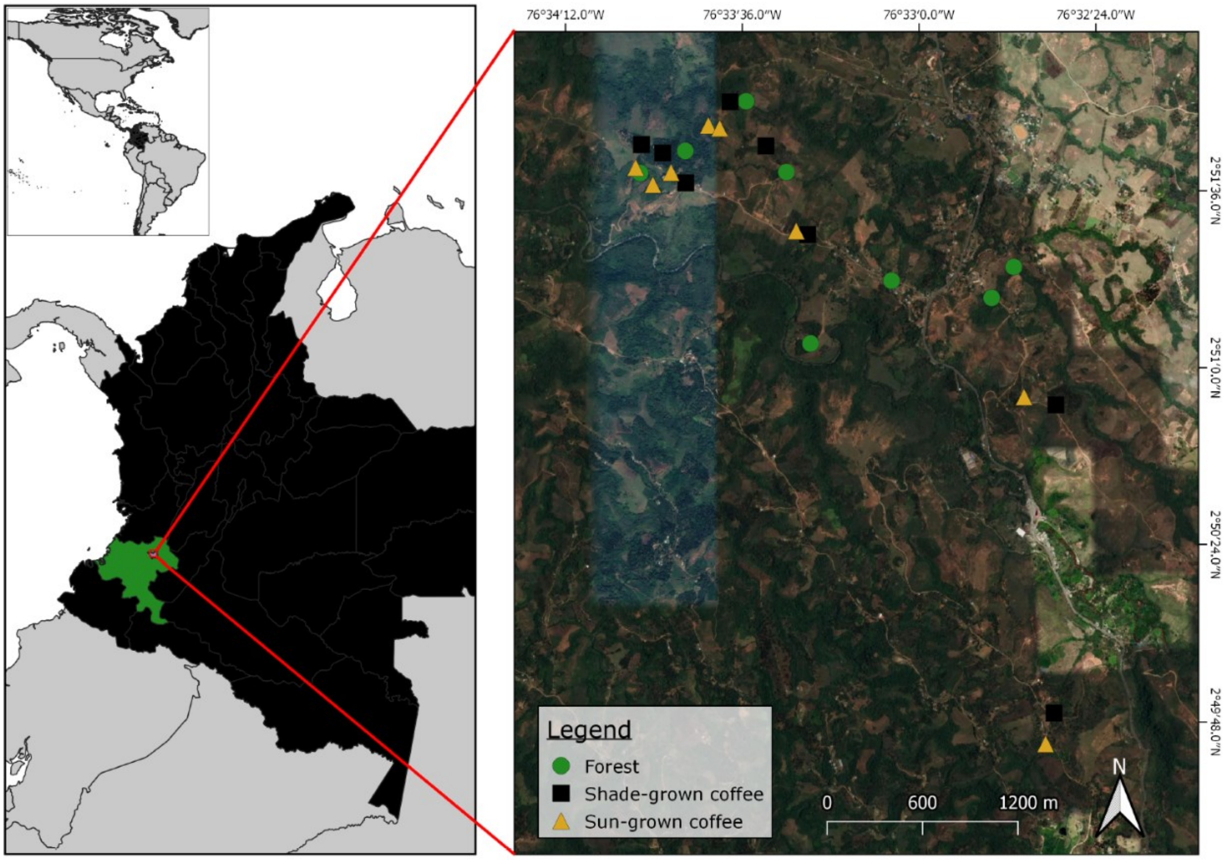


Figure 4.1. Distribution of sampling sites in the department of Cauca, Colombia.

4.3.2 Local and landscape environmental characteristics

To evaluate the effect of environmental variables that could contribute to biodiversity differences between management practices, we measured environmental variables at local and landscape scales. At local (plot-level) scale, we measured soil temperature and humidity, canopy cover and litter depth in all our sampled sites. Additionally, in sampled coffee plantations we measured number of twigs and litter volume below coffee bushes, coffee bush height, distance among coffee bushes and among rows as an estimate of crop density in each plantation. We characterized the landscape surrounding each site using geographic information system (GIS) approaches. We computed the maximum Normalized Difference Vegetation Index (NDVI) value from available Landsat images available in Google Earth Engine (Gorelick et al. 2017) for our sampling region. Using QGIS 3.14.15- Pi (QGIS.org 2020), we calculated the mean NDVI and its coefficient of variation within 50, 100, 250 and 500 m buffers for each site. We chose these buffer sizes following others who have studied landscape effects on ants (e.g., De la Mora et al.

2013, Iverson et al. 2019). See Appendix S4.2 for details on how we measured and processed these variables, and subsequently used them for supplementary analyses.

4.3.3 Ant community sampling

At each sampling site, we used five pitfall traps and five 1 m^2 leaf-litter extractions, each located 10 m apart, following a 50 m transect into the farm that started at least 10 m away from the edge of the plantation. Each pitfall trap consisted of a 266-ml cup filled with 90 ml of 70% ethanol, collected after 48 h. We hung five Winkler Extractors to air-dry for 48 h, inducing ants to fall to an ethanol-filled vial. Additionally, two researchers systematically examined coffee bushes, tree branches at and below eye-level, leaf-litter, under rocks and logs at each site for 30 min/researcher (adapted from Bestelmeyer et al. 2000). We sampled all sites twice, in August 2015 and between December 2015 and January 2016. We pooled this data for our main analyses. We conducted secondary analyses using data from each sampling campaign separately to account for any seasonal differences. All samples were transported to the Laboratorio de Ecología y Comportamiento de Insectos at Universidad del Valle in Cali, Colombia, for identification and permanent storage.

4.3.4 Community phylogenetics

To assess the phylogenetic structure of ant communities, we grafted current subfamily-level trees (Schmidt and Shattuck 2014, Ward et al. 2015, Blaimer et al. 2015b) onto a backbone, genus-level phylogeny (Moreau and Bell 2013), maximizing genera coverage. We pruned this tree to only include genera found in our samples. We excluded the genus *Rasopone* from our analyses because its phylogenetic placement remains unknown (Schmidt and Shattuck 2014). Based on our species list, we simulated 1000 species-level trees to account for phylogenetic uncertainty in later analyses (Fowler et al. 2014). We obtained species phylogenetic relationships from a Yule (pure-birth) process using the *genus.to.species.tree* function in the ‘phytools’ package for R (Revell 2012). We calculated a consensus tree using maximum clade credibility (Heled and Bouckaert 2013) using the *mcc* function in the ‘phangorn’ package (Schliep 2011). This method tallies the support of clades across the 1000-tree set and then selects the tree (Fig. 4.2) with the highest overall clade support (i.e., highest posterior probability).

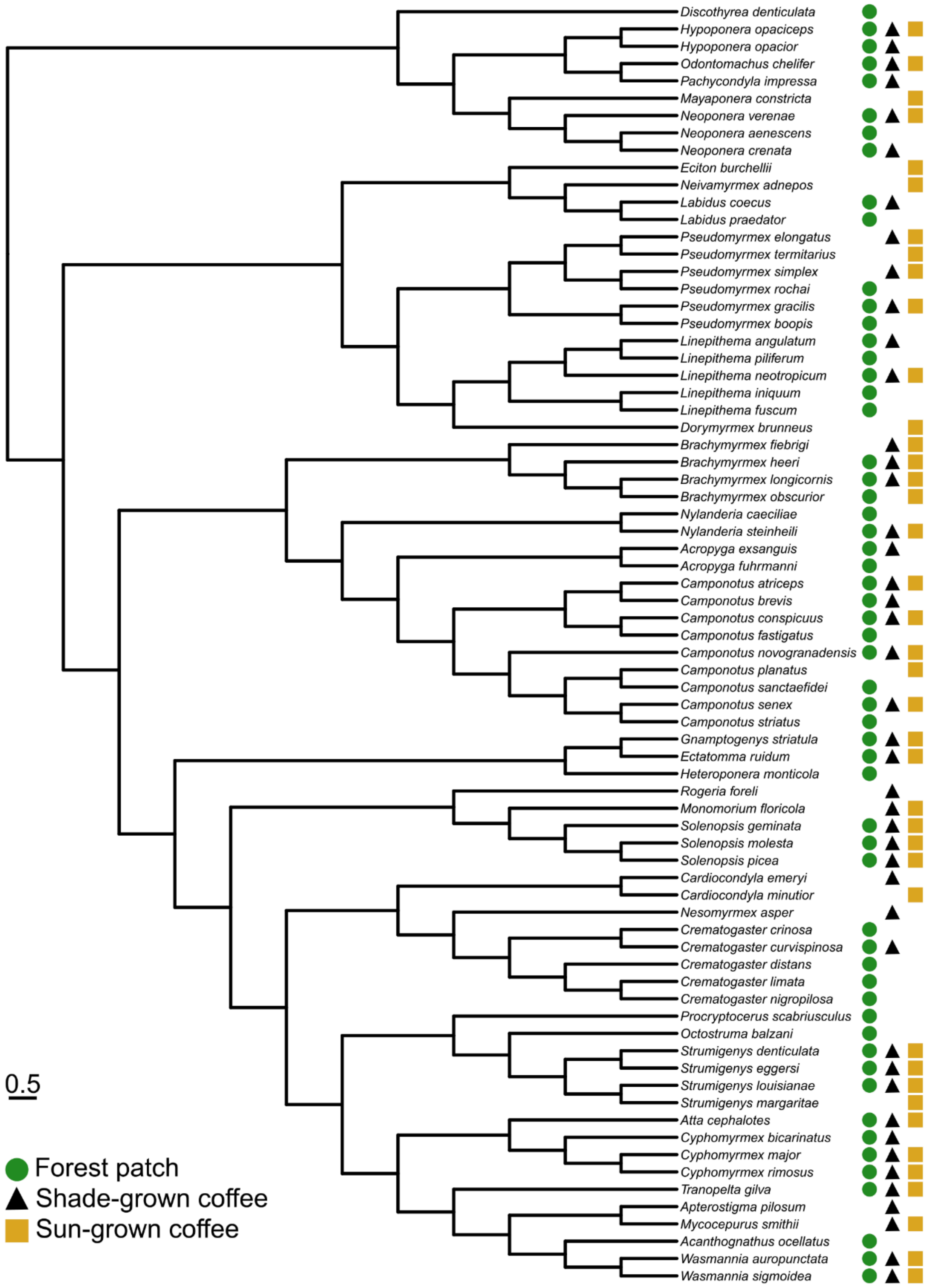


Figure 4.2. Dendrogram depicting the hypothesized phylogenetic relationships between species present in sampled sites. Calculated using Maximum Credibility Clade (MCC). Symbols represent the habitat in which a species occurred.

4.3.5 Functional traits

We used a combination of 12 morphological and life-history traits relevant to resource use and microhabitat preference (see Appendix S4.1). Due to the social structure of ants, we focused on traits at three levels: colony, worker, and queens. We hypothesized trait-specific responses to a reduction in canopy cover as a result of agricultural intensification (Brousseau et al. 2018, Andersen 2019). We assumed traits had no intra-specific trait variation. This omission likely results in a bias towards weaker trait-environment relationships (Hulshof and Swenson 2010, Jung et al. 2010). Thus, future work should include intra-specific variation in their analyses (Siefert et al. 2015).

We obtained species-specific trait values from taxonomic descriptions and specimen photographs from databases such as Antweb (www.antweb.org) and AntWiki (www.antwiki.org; see full reference list in Appendix S4.1). We complemented life-history information from observations made in the field by our research group. To measure morphological traits from photographs, we used the software ImageJ v1.51j8 (Schneider et al. 2012) following the protocol described by Parr et al. (2017).

4.3.6 Statistical analyses

4.3.6.1 Functional traits

To quantity the functional structure of ant communities we relied on a trait dendrogram. We first log-transformed and scaled our trait data. We then used this data in a Principal Coordinate Analysis (PCoA) to produce orthogonal axes of function. From these, we selected the first 6 axes that explained ~90% (Villéger et al. 2008). These calculations were done using the *dbFD* function of the 'FD' package for R (Laliberté et al. 2014). We converted these trait axes into a Euclidean distance matrix (*dist* function) that we used to produce a dendrogram representing the functional relationship among species using the UPGMA (*hclust* function) method (Mouchet et al. 2010).

4.3.6.2 Compositional dissimilarity between habitats

To investigate the compositional differences between nearby forest patches and coffee plantation management strategies, we calculated a dissimilarity matrix based on the Simpson pair-wise index (Simpson 1943, Lennon et al. 2001). This metric generally reflects the compositional differences due to species turnover (replacement), while accounting for differences in species richness between sites (Baselga 2010). To calculate this index for all diversity components of interest, we used the functions *beta.pair* and *phylo.beta.pair* from the 'betapart' package (Baselga and Orme 2012). To visualize dissimilarities, we generated nonparametric multidimensional scaling (NMDS) plots via the *metaMDS* function in the 'vegan' package for R (Oksanen et al. 2019). We tested the differences in the taxonomic, phylogenetic, and functional position of the community centroids (multivariate location) between forest and both coffee plantation management practices via "Permutational Multivariate Analysis of Variance" (PERMANOVA) using the *adonis2* function in the 'vegan' package.

4.3.6.3 Compositional dispersion within habitats

To evaluate the degree of biotic homogenization in each habitat type, we quantified the distance to centroid (multivariate dispersion) of ant communities residing in forests and both types of coffee plantations through a "Permutational Analysis of Multivariate Dispersion" (PERMDISP) using the *betadisper* function in the 'vegan' package.

4.3.6.4 Standardized effect sizes

To allow for comparisons between habitat types, we calculated a standardized effect size (SES) of multivariate dispersion ($D_{centroid}$) for each community and diversity dimension using the following equation:

Equation 4.1:

$$\text{SES } D_{centroid} = \frac{\text{Mean}_{obs} - \text{Mean}_{null}}{\text{SD}_{null}}$$

We calculated 1000 null $D_{centroid}$ values for each observed $D_{centroid}$ value (Swenson 2014). For this, we generated 1000 null communities using the "Independent swap" algorithm found in the *randomizeMatrix* function of the 'picante' package (Kembel et al. 2010). We then calculated the taxonomic, phylogenetic, and functional multivariate dispersion on each of these null communities using the method described in the previous paragraph and used them to calculate the null $D_{centroid}$ values. Finally, we used a one-sample t-test (*t.test* function) to investigate whether average SES $D_{centroid}$ for each habitat differed from zero (null expectation). All analyses were performed using R (R Core Team 2020).

4.3.6.5 Influence of spatial and environmental factors on ant communities

To test for spatial autocorrelation of our sampling sites, we performed a Mantel test with the *mantel.test* function of the 'ade4' package for R (Dray and Dufour 2007). We found that spatial distance and land use type were not correlated ($r = -0.04$, $P = 0.70$, 9999 replicates). This result allowed us to reject the spatial distribution of the sampled farms and forest patches as a confounding factor for our interpretation.

To analyze the effect of environmental variables on the compositional differences of ant assemblages, we performed a PERMANOVA as described above. To account for the multicollinearity among our measured environmental variables, we performed PCAs using the *prcomp* function in R (R Core Team 2020) and produced a biplot to visualize the summarized information (Fig. 4.3A). We then used these orthogonal axes that describe local and landscape environmental variables as independent variables in the PERMANOVA (see Appendix S4.3, Table S4.3a).

4.4 Results

We collected a total of 25,865 individual workers belonging to 75 ant species from 9 subfamilies and 36 genera across the 24 sites. Of these, 28% species were found exclusively in forest patches, ~7% exclusively in shade-grown coffee plantations, and ~11% exclusively in sun-grown coffee plantations. There was some overlap in species occurrence; however, only 35% species occurred in all three land-use types (see Appendix S4.3, Fig. S4.3a). Species accumulation curves confirmed the adequacy of our species sampling (see Appendix S4.3, Fig. S4.3b).

4.4.1 Local and landscape environmental differences between habitats

While we found significant differences in the local environmental factors between habitats, we found no significant differences at the landscape scale (Fig. 4.3; see Appendix S4.2, Fig. S4.2b). More specifically, we found that soil humidity ($F= 35.73$, $df= 2$, $p < 0.001$) and litter depth ($F= 18.23$, $df= 2$, $p < 0.001$) are higher in forest patches and shade-grown plantations. Additionally, shade-grown plantations had higher crop density (distance between planted rows; $F=5.99$, $df= 1$, $p = 0.02$), number of twigs ($F=21.54$, $df = 1$, $p < 0.001$) and leaf-litter volume ($F= 9.04$, $df= 1$, $p < 0.01$).

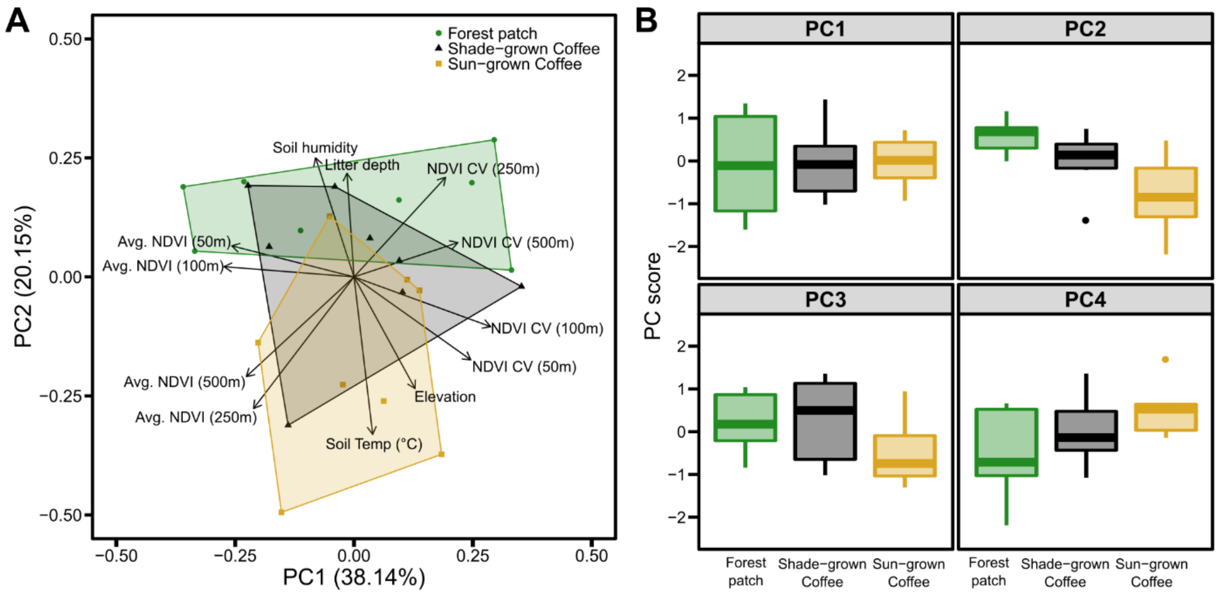


Figure 4.3. PCA of environmental variables measured in all habitat types. (A) PCA biplot depicting the Euclidean distances between the sampled sites and the eigenvectors of environmental variables measured in all habitat types. Different colored symbols represent different habitat types with green circles, black triangles, and golden squares representing nearby forest patches, shade-grown coffee plantations, and sun-grown coffee plantations, respectively. (B) Boxplots depicting the scores of sampled sites for each significant PC axis. The box encloses the 25–75th percentiles of the values, bold lines represent median and the whiskers extend to 1.5 times the interquartile range. See Appendix S2, Table S2b for contributions to each PC axis for each environmental variable.

4.4.2 Compositional differences between forest patches and management strategies

The taxonomic ($F = 7.31$, $p < 0.001$, $R^2 = 0.41$), phylogenetic ($F= 9.04$, $p < 0.01$, $R^2 = 0.46$) and functional trait composition ($F= 7.06$, $p < 0.001$, $R^2 = 0.41$) differed between habitat types (Fig. 4.4A-C, Table 4.1). When analyzing the data separately, we observed significant differences in ant composition across biodiversity dimensions in both seasons (Table 4.1). Specifically, pairwise analyses for each sampling campaign revealed that there were no significant differences between the taxonomic, phylogenetic, and functional compositions of shade-grown plantations and that of nearby forest patches (Table 4.1).

Table 4.1. Summary of statistics for PERMANOVAs of the effect of habitat type on taxonomic, phylogenetic, and taxonomic compositional turnover of ant communities. Significant values ($p < 0.05$) are highlighted in bold.

	POOLED			**AUGUST**			**DECEMBER-JANUARY**		
	F	**R^2**	**p-value**	**F**	**R^2**	**p-value**	**F**	**R^2**	**p-value**
Taxonomic	7.301	0.411	< **0.001**	6.251	0.373	< **0.001**	5.139	0.328	< **0.001**
Forest/Shade-grown	3.422	0.196	**0.003**	2.068	0.129	**0.044**	2.881	0.171	**0.012**
Forest/Sun-grown	9.095	0.394	< **0.001**	8.598	0.381	< **0.001**	6.416	0.314	< **0.001**
Shade-grown/Sun-grown	11.821	0.458	< **0.001**	10.111	0.419	< **0.001**	7.015	0.334	< **0.001**
Phylogenetic	9.037	0.463	< **0.001**	6.312	0.375	< **0.001**	5.812	0.356	< **0.001**
Forest/Shade-grown	4.279	0.234	**0.002**	2.379	0.145	0.061	1.829	0.116	0.175
Forest/Sun-grown	14.081	0.501	< **0.001**	10.351	0.425	< **0.001**	8.321	0.373	< **0.001**
Shade-grown/Sun-grown	10.235	0.422	< **0.001**	8.002	0.364	< **0.001**	8.338	0.373	< **0.001**
Functional	7.061	0.402	< **0.001**	4.981	0.322	< **0.001**	4.354	0.293	< **0.001**
Forest/Shade-grown	3.581	0.204	**0.002**	1.319	0.086	0.332	1.859	0.117	0.192
Forest/Sun-grown	9.302	0.399	< **0.001**	6.221	0.308	< **0.001**	4.561	0.246	**0.004**
Shade-grown/Sun-grown	9.349	0.401	< **0.001**	9.115	0.394	< **0.001**	8.879	0.388	< **0.001**

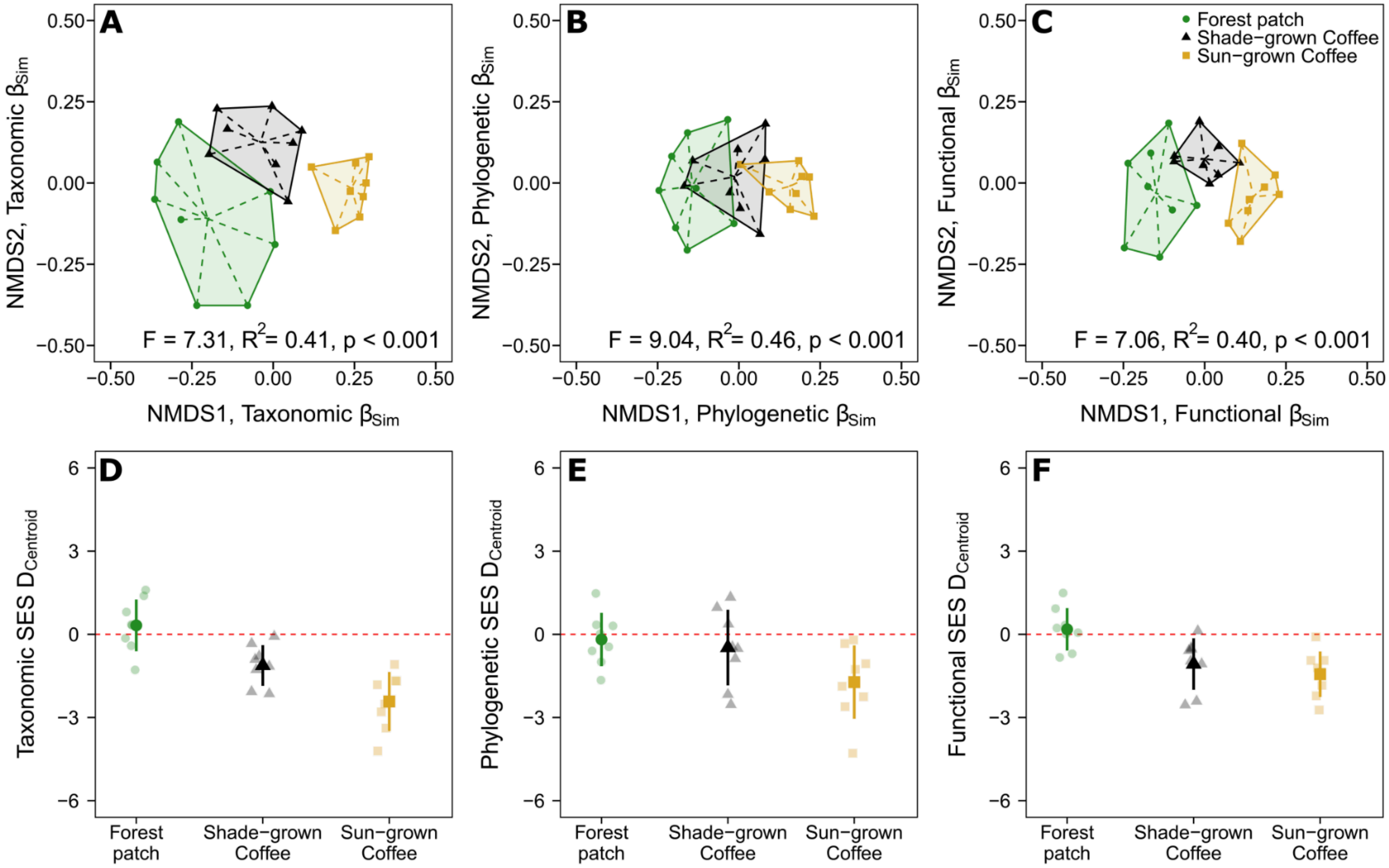


Figure 4.4. Ant community composition analyses between pairs of local communities in each environment. (A, B, C) NMDS ordination of sites within forest and coffee plantations based on taxonomic, phylogenetic, and functional Simpson's dissimilarity index. (D, E, F) Scatterplot of mean ± SD of standardized effect size of the distance to multivariate space centroid (SES $D_{centroid}$) of each site. Red dashed line represents null expectation.

4.4.3 Biotic homogenization of ant communities in coffee plantations

The taxonomic (F = 11.47, df= 2, $p < 0.01$), phylogenetic (F = 4.44, df =2, p = 0.02) and functional (F = 5.71, df =2, p = 0.01) multivariate dispersion (i.e., homogenization) differed significantly between the habitat types (Fig. 4.4D-F). We found that homogenization patterns were consistent with null expectations across diversity dimensions in forest patches ($p > 0.05$, one-sample *t* test). Our analysis detected ant communities found in shade-grown plantations were taxonomically (t = -4.3, df = 7, p = 0.03) and functionally (t = -3.26, df = 7, p = 0.01) less homogeneous than expected, but not phylogenetically (t = -0.99, df = 7, p = 0.35). In contrast, ant communities found in sun-grown coffee plantations were more homogenous than null expectations across diversity dimensions ($p < 0.05$).

4.5 Discussion

Our results indicate that management practices of shade-grown coffee plantations can lessen the effects of anthropogenic disturbance on ant diversity when compared to intensified unshaded monocultures. As hypothesized, ant communities found in shade-grown coffee plantations neighbouring forest patches were more similar across diversity dimensions but differed from those found in sun-grown coffee plantations. Additionally, we observed high levels of redundancy in ant composition in unshaded monocultures. Finally, we discuss the environmental factors by which land management practices alter ant diversity.

4.5.1 Compositional dissimilarity of ant communities

Shade-grown coffee practices appear to buffer the effect of coffee growing on ant communities. The taxonomic composition of ant communities in shade-growing plantations was more similar to those in nearby forest patches than to those in sun-grown coffee plantations. One might expect these compositional differences to be driven by the presence of arboreal ants typical of natural forest patches in shade-grown but not in sun-grown coffee plantations. However, we observed a similar pattern when excluding arboreal species from our analyses (see Appendix S4.4, Fig. S4.4a, Tables S4.4a-b). Furthermore, we found habitat generalists occurring ubiquitously in all environments, matching previous studies (Gallego Ropero 2005, Armbrecht et al. 2005, Urrutia-Escobar and Armbrecht 2013, Cuautle et al. 2016). We found 9 species occurring in both forest patches and shade-grown plantations, and only 1 species occurring in both forest patches and sun-grown plantations. Ant communities found in sun-grown plantations were also phylogenetically distinct from both shade-grown plantations and forest patches. While arboreal genera (e.g., *Camponotus*, *Crematogaster*) occurred mostly in shade-grown plantations and forest patches, sun-grown plantations mainly harbored soil-dwelling ants (Fig. 4.2). This pattern indicates that ants occurring in these habitats have distinct evolutionary histories and relationships (Lucky et al. 2013). Most ant lineages present in forest patches and shade-grown plantations were associated with more humid and closed canopy environments, pointing to their convergent habitat of origins and evolutionary history (Lessard et al. 2012, Vasconcelos et al. 2018, Economo et al. 2018).

Consistent with previous studies, we showed that land conversion and agricultural intensification can alter ant functional diversity and composition (Armbrecht et al. 2005, Urrutia-Escobar and Armbrecht 2013, Liu et al. 2016). Specifically, we found that ants are smaller in shade-grown coffee plantations and are lighter-colored in both shade-grown and sun-grown coffee plantations compared to those found in forest patches (see Appendix S4.5). Smaller workers are able to navigate more complex habitats (Kaspari and Weiser 1999) such as the leaf litter layer present in shade-grown plantations (Armbrecht et al. 2005). On the other hand, a light-coloured cuticle absorbs heat more slowly than dark-colored cuticles (Clusella Trullas et al. 2007). Cuticle lightness has been linked to the ability of species to adapt to altered thermal conditions (Bishop et al. 2016, Law et al. 2019), potentially allowing workers to forage closer to their maximum thermal limits (Cerdá et al. 2013).

4.5.2 The effect of management on environmental conditions and compositional dissimilarity

While local environmental conditions differ between habitat and management types, the landscape matrix surrounding farms does not vary significantly (see Appendix S4.2, Fig. S4.2b). Shade-grown plantations share more environmental similarities with forest patches than with sun-grown plantations. Besides having trees providing shade to the forest floor, shade-grown plantations have similarly deep and damp leaf-litter relative to sun-grown plantations (Fig. 4.3; Appendix S4.2, Fig. S4.2b). These differences in soil characteristics drive compositional differences between habitat types (see Appendix S4.3, Table S4.3a), which corroborate results from previous studies in Colombian coffee plantations (e.g., Armbrecht et al. 2006).

Management-related variables differ significantly between shade-grown and sun-grown coffee plantations. Shade-grown plantations have a higher crop density (see Appendix S4.2, Fig. S4.2b-c), number of twigs and leaf-litter volume than sun-grown plantations, which was associated with distinct ant communities (see Appendix S4.3, Table S4.3a). The more dense and diverse vegetation found in shade-grown plantations may enhance resource (e.g., nesting sites, prey) availability and diversity, and in turn, increase ant diversity (Ashraf et al. 2018). Previous work with ants conducted in similar systems also showed that high leaf-litter volume and number of twigs could increase the richness of native ants (Armbrecht and Perfecto 2003, De la Mora et al. 2013).

4.5.3 Biotic homogenization of ant communities

We found low compositional dispersion (i.e., high redundancy) between sun-grown coffee plantations and across diversity dimensions. Besides increasing habitat openness, intensive management practices limit the types of habitats and feeding resources needed by specialist ants (Philpott and Foster 2005). Sun-grown plantations contained more taxonomically, phylogenetically, and functionally homogenous ant communities. These communities were mainly comprised of a subset of generalist species found in forest patches or shade-grown plantations. Additionally, we found at least twice as many unique species exclusive to forest habitats compared to both types of management practices (Appendix S4.3, Fig. S4.3a). In ant communities, rare species are often cryptic specialized predators nesting in the leaf litter (Philpott and Armbrecht 2006, Lessard et al. 2007) and therefore providing unique and targeted services.

Agricultural intensification produces more homogeneous habitat, which favors subsets of closely related and morphologically similar species (Armbrecht et al. 2005, Morelli et al. 2016). The loss of closely related specialized ant predators in response to various forms of disturbance could be a general pattern (Lessard et al. 2009). Therefore, these ecosystems could be less stable, less resilient, and vulnerable to extinction and should be properly evaluated to avoid the loss of ecosystem functions (Naeem et al. 1994, Oliver et al. 2015). Currently, our knowledge of the role of ants in agroecosystems is still limited to pest control and subsequent increased yield (Philpott and Armbrecht 2006, De la Mora et al. 2015, Morris et al. 2018). However, their role as seed dispersers and nutrient cyclers (Del Toro et al. 2012 and references therein) points to a potential greater importance in agroecosystems (Wielgoss et al. 2014).

4.6 Conclusion

Improving our understanding of the effects of agricultural activities on biodiversity is a critical subject of agroecology and conservation. Our results highlight how polyculture management practices lessen the negative effects of intensive practices across multiple biodiversity dimensions. Furthermore, our environmental analyses allowed us to identify that shade-grown plantations maintain similar environmental conditions to forest patches. Thus, farm managers should strive for forest-like leaf-litter conditions to help mitigate loss across diversity dimensions. Additionally, maintaining, or augmenting leaf-litter depth and twig number below coffee bushes could be used as a strategy to conserve ant predation services by favouring the persistence of specialized arboreal predators that can act as biocontrol agents. These practices would not only benefit ant diversity, but numerous additional benefits may come indirectly from ant conservation-oriented practices.

4.7 Supplementary materials

APPENDIX S4.1. Supplementary information of trait measurements

Supplementary Table S4.1a. Description of ant traits examined in this study and their hypothesized functional response.

Level	Trait	Measurement unit	Hypothesized function	Hypothesized response to decreased canopy cover
Colony	Polymorphism	0-Monomorphic 1-Polymorphic	Relates to range of functional roles performed (Mertl and Traniello, 2009)	Ant colonies will be mostly monomorphic
	Queen number	0-Monogynous 1-Polygynous	Influences growth rate, competitive ability, and efficiency (Rosset and Chapuisat, 2007)	Ant colonies will have a higher number of queens
	Number of nests	0-Monodomous 1-Polydomous	Having multiple nests confers competitive advantage (McGlynn, 1999)	Ant colonies will be mostly polydomous
	Diet	1- Generalist 2- Generalist predator 3- Generalist seed harvester 4- Generalist sugar feeder 5- Seed harvester 6- Specialist predator 7- Fungivore	Related to trophic position (Hölldobler and Wilson, 1990)	Ant colonies will be mostly generalist
Worker	Body size	Weber’s length (mm)	Strongly correlates with many physiological traits (Gibb et al., 2015)	Ant workers will have larger body sizes

Level	Trait	Measurement unit	Hypothesized function	Hypothesized response to decreased canopy cover
	Head size	Head width/Head length (mm)	Indicative of foraging substrate and diet (Weiser and Kaspari, 2006)	Ant workers will have larger heads
	Eye size	Maximum eye width (mm)	Indicative of food searching behavior and activity times (Weiser and Kaspari 2006)	Ant workers will have larger eyes
	Hind femur length	Maximum length (mm)	Relates to habitat complexity adaptation (Schofield et al., 2016)	Ant workers will have longer legs
	Pilosity	Count of hairs crossing mesosoma profile	Increases protection against dehydration (Wittlinger et al., 2007)	Ant workers will have an increased number of hairs
	Dominant color	1- Black 2- Dark-brown 3- Light-brown 4- Yellow	Related to thermal melanism hypothesis and environmental stress (Clusella Trullas et al., 2007)	Ant workers will be lighter
Reproductive	Body size	Weber’s length (mm)	Strongly correlates with many physiological and colony traits (Wiernasz and Cole, 2003)	Ant queens will have larger body sizes
	Pronotum width	Pronotum longitudinal length in dorsal view (mm)	Influences colony’s survivability and fitness (specially recently founded). Can be used as indicator dispersal distance capabilities (Wiernasz and Cole 2003)	Ant queens will have wider pronotums

Supplementary Table S4.1b. References and specimens used for trait measurements of each species found in the study sites.

Subfamily	Species	ANTWEB specimen	Reference
Proceratiinae	*Discothyrea denticulata*	CASENT0178697	Weber 1939
Ponerinae	*Hypoponera opaciceps*	CASENT0005435	Smith 1936
	Hypoponera opacior	CASENT0005436	Smith 1936
	Mayaponera constricta	CASENT0217555	Mackay and Mackay 2010
	Neoponera aenescens	CASENT0249127	Mackay and Mackay 2010
	Neoponera crenata	CASENT0178178	Mackay and Mackay 2010
	Neoponera verenae	CASENT0103061	Mackay and Mackay 2010
	Odontomachus chelifer	CASENT0173536	De la Mora et al. 2016
	Pachycondyla impressa	CASENT0178689	Mackay and Mackay 2010
	Rasopone ferruginea	CASENT0249143	Mackay and Mackay 2010
Dorylinae	*Eciton burchellii*	CASENT0249452	Wild 2007
	Labidus coecus	CASENT0173511	Wetterer and Snelling 2015
	Labidus praedator	CASENT0173515	Barth et al. 2015
	Neivamyrmex adnepos	CASENT0249470	Borgmeier 1955
Dolichoderinae	*Dorymyrmex brunneus*	CASENT0192705	Cuezzo and Guerrero 2011
	Linepithema angulatum	CASENT0249737	Wild 2007
	Linepithema fuscum	CASENT0106976	Wild 2007

Subfamily	Species	ANTWEB specimen	Reference
	Linepithema iniquum	CASENT0106982	Wild 2007
	Linepithema neotropicum	CASENT0104903	Wild 2007
	Linepithema piliferum	CASENT0106979	Wild 2007
Pseudomyrmicinae	*Pseudomyrmex boopis*	INBIOCRI001281684	Gillette et al 2015
	Pseudomyrmex elongatus	CASENT0005874	Gillette et al 2015
	Pseudomyrmex gracilis	CASENT0173763	Ward 1993
	Pseudomyrmex rochai	CASENT0281898	Kempf 1972
	Pseudomyrmex simplex	CASENT0104281	Gillette et al 2015
	Pseudomyrmex termitarius	CASENT0173786	Mill 1981
Formicinae	*Acropyga exsanguis*	CASENT0249918	LaPolla 2004
	Acropyga fuhrmanni	CASENT0909901	LaPolla 2004
	Brachymyrmex fiebrigi	CASENT0173476	Ortiz-Sepulveda et al. 2019
	Brachymyrmex heeri	CASENT0173478	Ortiz-Sepulveda et al. 2020
	Brachymyrmex longicornis	USNMENT00757156	Ortiz-Sepulveda et al. 2021
	Brachymyrmex obscurior	CASENT0104889	Ortiz-Sepulveda et al. 2022
	Camponotus atriceps	CASENT0178616	De la Mora et al. 2015
	Camponotus brevis	INBIOCRI001282798	Longino 2010
	Camponotus conspicuus	CASENT0217634	Longino 2010

Subfamily	Species	ANTWEB specimen	Reference
	Camponotus fastigatus	CASENT0173417	Longino 2010
	Camponotus novogranadensis	CASENT0173434	Longino 2010
	Camponotus planatus	CASENT0103700	Longino 2010
	Camponotus sanctaefidei	CASENT0173447	Longino 2010
	Camponotus senex	CASENT0280095	Longino 2010
	Camponotus striatus	CASENT0903642	Longino 2010
	Nylanderia caeciliae	CASENT0903137	Longino 2010 (appears as *Paratrechina caeciliae*)
	Nylanderia steinheili	CASENT0178613	Lapolla and Kallal 2019
Ectatomminae	*Ectatomma ruidum*	CASENT0178705	Kugler and Brown 1982
	Gnamptogenys striatula	CASENT0173386	Lattke 1995
Heteroponerinae	*Heteroponera monticola*	CASENT0902410	Kempf and Brown 1970
Myrmicinae	*Acanthognathus ocellatus*	CASENT0178718	Fernandez et al. 1996
	Apterostigma pilosum	CASENT0173821	Wild 2007
	Atta cephalotes	CASENT0173617	Wells et al. 2017
	Cardiocondyla emeryi	CASENT0173259	Creighton and Snelling 1974
	Cardiocondyla minutior	CASENT0103436	Seifert 2003
	Crematogaster crinosa	CASENT0173935	Longino 2010
	Crematogaster curvispinosa	CASENT0173308	Longino 2010

Subfamily	Species	ANTWEB specimen	Reference
	Crematogaster distans	CASENT0103431	Longino 2010
	Crematogaster limata	CASENT0912774	Longino 2010
	Crematogaster nigropilosa	CASENT0173945	Longino 2010
	Cyphomyrmex bicarinatus	CASENT0901672	Snelling and Longino 1992
	Cyphomyrmex major	CASENT0909379	Snelling and Longino 1992
	Cyphomyrmex rimosus	CASENT0173243	Snelling and Longino 1992
	Monomorium floricola	CASENT0173274	Heterick 2006
	Mycocepurus smithii	CASENT0179467	Mackay et al. 2004
	Nesomyrmex asper	CASENT0173991	Longino 2010
	Octostruma balzani	CASENT0178665	Longino 2013
	Procryptocerus scabriusculus	CASENT0106043	Longino and Snelling 2002
	Rogeria foreli	CASENT0006150	Kugler 1994
	Solenopsis geminata	CASENT0104522	Trager 1991
	Solenopsis molesta	CASENT0005805	Pacheco and Mackay 2013
	Solenopsis picea	CASENT0904627	Pacheco and Mackay 2013
	Strumigenys denticulata	CASENT0178117	Bolton 2000
	Strumigenys eggersi	CASENT0103845	Bolton 2000
	Strumigenys louisianae	CASENT0003321	Bolton 2000

Subfamily	Species	ANTWEB specimen	Reference
	Strumigenys margaritae	CASENT0104473	Bolton 2000
	Tranopelta gilva	CASENT0010793	Fernandez 2003
	Wasmannia auropunctata	CASENT0005064	Longino and Fernandez 2007
	Wasmannia sigmoidea	CASENT0901665	Longino and Fernandez 2007

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APPENDIX S4.2. Environmental analyses

Methods

To characterize the local (plot-level) scale environmental characteristics, we measured soil temperature and humidity, canopy cover and litter depth at each sampling location. We measured all these environmental variables next to each pitfall trap and leaf-litter extraction points, following the same 50 m-long transect we used for the ant community sampling. More specifically, we measured soil humidity at ~10cm depth using Kelway® Soil pH and Moisture Meter (Model HB-2). We measured canopy cover using a GRS DensitometerTM at 10 random points within a 1 m^2 subplot next to each sampling point. We measured litter-depth using a centimeter ruler at four random points within a 30 cm radius from each pitfall trap and at each corner of the 1 m^2 leaf-litter extraction subplots. We then averaged each environmental variable across all measurement points to establish a single value per plot. Finally, our soil hygrometer broke before we could measure the last 2 forest plots (Forest7 and Forest8), 1 shade-grown coffee (Shade8) plantation and 1 sun-grown coffee plantation (Sun8). Thus, we imputed the missing values by substituting them with the average value for the habitat type to avoid the loss of valuable sample sites (van Buuren 2012).

In addition to the previous local scale environmental characteristics, we measured the number of twigs and leaf-litter volume below coffee bushes (within a 4 m^2 quadrat with the coffee bush as the center). We also obtained the distance between coffee bushes and between planted rows in both types of plantations. These previous measurements give us an idea of the crop density in each coffee plantation and planting strategies for each plantation. Though we only had access to these measurements in May 2017, the plantation owners assured us they did not change their management practices between our sampling period and when we had access to these measurements. We could not obtain these measurements for 5 plantations (Sun5, Sun7, Shade4, Shade6 and Shade7), thus, we imputed these missing values by using the same method described in the previous paragraph.

We characterized the landscape surrounding each site using geographic information system (GIS) approaches. We computed the maximum Normalized Difference Vegetation Index (NDVI) value from available Landsat images available in Google Earth Engine (GEE; Gorelick et al. 2017) for our sampling region. To do this, we made a composite of the available Landsat 8 images between 01-AUG-2015 and 31-JUL-2016, using the B5 (near infra-red) and B4 (red) bands of the images. We then calculated NDVI by using the following formula for each pixel value:

$$NDVI = \frac{Band\ 5 - Band\ 4}{Band\ 5 + Band\ 4}$$

With the resulting composite image, we selected the maximum NDVI value to account for the discontinuities in Landsat paths due to differences in times of passing and presence of clouds. With this, we obtained the Landsat 8 greenest pixel composite (Fig. S4.2a). Then, using QGIS 3.14.15-Pi (QGIS.org, 2020), we calculated for each site the mean NDVI and its coefficient of variation within 50, 100, 250, 500 and 1000 m radius from the sampling coordinates.

We tested for environmental differences between habitat types by running individual one-way ANOVAs for each variable as a response variable (Fig. S4.2b). Then, to account for the multicollinearity among our measured environmental variables, we first standardized (mean of 0 and SD of 1) our data and then performed PCAs using the *prcomp* function in R. In these analyses, we included elevation as a surrogate for other environmental variables correlated with elevation (e.g. temperature, humidity). We also excluded canopy cover because all sun-grown plantations had a value of zero and could skew the PCA results. After performing the analyses, we extracted the contribution of each environmental variable to each PC (Tables S4.2b-c). To visualize the results, we produced biplots of both PCAs (Fig. 4.3 and S4.42c). Finally, to investigate the effects of these environmental variables in compositional differences between habitats, we included the first 4 and the first 5 PCs of each analyses into a PERMANOVA (see Appendix S4.3, Table S4.3a) because they were significant based on the Kaiser-Guttman criterion (Jackson 1993).

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Supplementary Table S4.2a. Geographic location and general information of sampled sites and farms.

Land use type	Sample code	Geographic location	Area (ha)	Altitude (masl)	Owner
Forest patch	Forest1	2°51′39.8″N, 76°33′27.0″W	1	1415	Caldono municipality
	Forest2	2°51′39.6″N, 76°33′56.8″W	0.5	1419	Ramiro Castillo
	Forest3	2°51′44.1″N, 76°33′47.6″W	0.5	1404	Maximiliano Ortiz
	Forest4	2°51′54.2″N, 76°33′35.2″W	0.5	1357	Manuel Vidal
	Forest5	2°51′17.7″N, 76°33′05.6″W	1.5	1479	Gerardo Peñaranda
	Forest6	2°51′14.2″N, 76°32′45.2″W	1	1449	Caldono municipality
	Forest7	2°51′04.9″N, 76°33′22.1″W	1	1336	Caldono municipality
	Forest8	2°51′20.5″N, 76°32′40.7″W	0.5	1428	Caldono municipality
Shade-grown coffee plantation	Shade1	2°51′37.6″N, 76°33′47.5″W	1	1435	Clara Ramos
	Shade2	2°51′45.4″N, 76°33′56.5″W	0.5	1437	Lilia González
	Shade3	2°51′45.1″N, 76°33′31.2″W	3	1436	Segundo Castillo
	Shade4	2°51′54.1″N, 76°33′38.5″W	2	1367	Manuel Vidal
	Shade5	2°51′27.1″N, 76°33′22.7″W	0.5	1479	Antonio Velasco
	Shade6	2°50′52.4″N, 76°32′32.1″W	6	1479	Armando Vélez

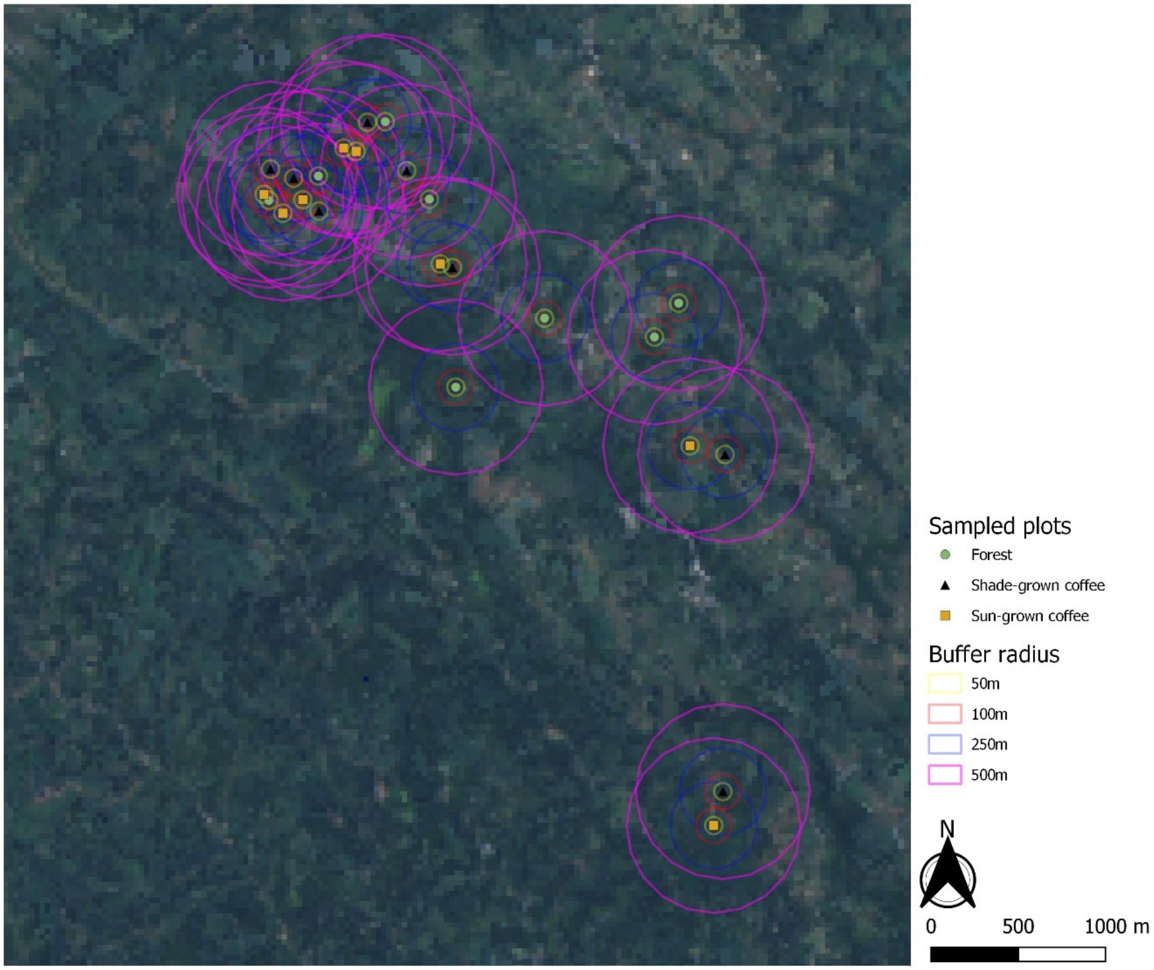
Land use type	Sample code	Geographic location	Area (ha)	Altitude (masl)	Owner
	Shade7	2°49′49.8″N, 76°32′32.5″W	1	1536	José Daza
	Shade8	2°51′43.7″N, 76°33′52.2″W	2	1419	Maximiliano Ortiz
Sun-grown coffee plantation	Sun1	2°51′39.7″N, 76°33′50.5″W	1	1438	Yaneth Ramos
	Sun2	2°51′49.3″N, 76°33′42.9″W	0.5	1408	Marino Muñoz
	Sun3	2°51′48.7″N, 76°33′40.6″W	0.5	1420	Segundo Castillo
	Sun4	2°51′40.7″N, 76°33′57.7″W	1	1425	Ramiro Castillo
	Sun5	2°51′27.8″N, 76°33′25.0″W	0.5	1489	Gerardina Velasco
	Sun6	2°50′54.0″N, 76°32′38.6″W	1	1486	Víctor Manzano
	Sun7	2°49′43.5″N, 76°32′34.2″W	8	1538	Fidel Melo
	Sun8	2°51′37.2″N, 76°33′54.2″W	1	1423	Jairo Morales

Supplementary Table S4.2b. Contributions of environmental variables measured in all land types (i.e., Forest patches, shade-grown and sun-grown plantations) for significant PC axes. Highest absolute values are highlighted in bold.

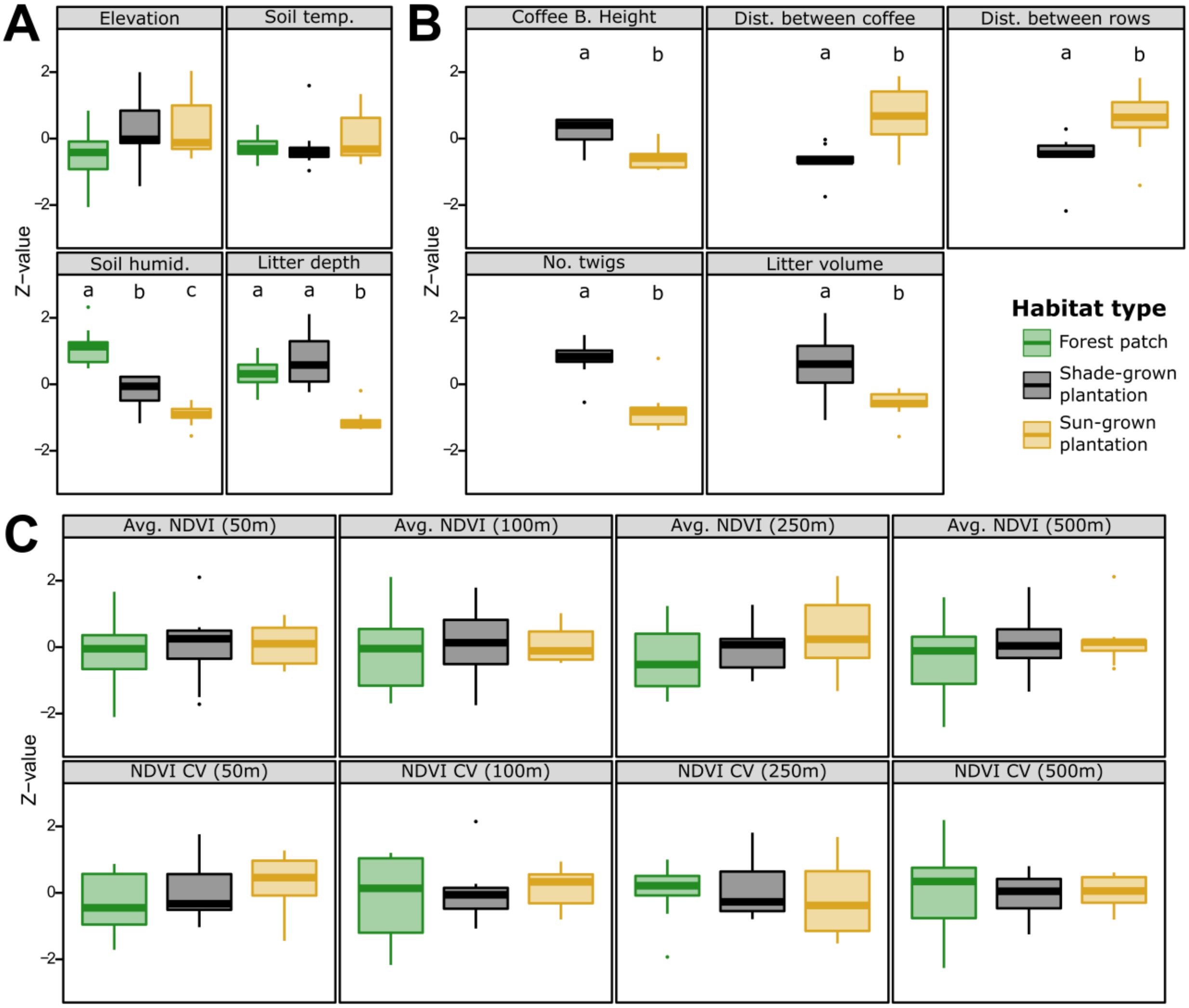
Environmental variable	**PC1**	**PC2**	**PC3**	**PC4**
Local				
Elevation	0.462	-0.613	0.602	0.370
Soil Temperature (°C)	0.141	**-0.865**	0.429	0.058
Soil humidity	-0.291	0.654	0.508	-0.503
Litter depth	-0.053	0.569	**0.803**	-0.264
Landscape				
Average NDVI				
50m radius	-0.926	0.171	0.061	**0.612**
100m radius	-0.983	0.056	0.231	0.457
250m radius	-0.760	-0.723	0.110	-0.040
500m radius	-0.815	-0.548	-0.245	-0.172
NDVI Coefficient of Variation				
50m radius	0.883	-0.455	0.097	-0.180
100m radius	**1.034**	-0.273	-0.106	-0.140
250m radius	0.693	0.547	-0.319	0.494
500m radius	0.786	0.188	0.335	0.596

Supplementary Table S4.2c. Contributions of environmental variables measured in coffee plantations (i.e., Shade-grown and sun-grown) for significant PC axes. Highest absolute values are highlighted in bold.

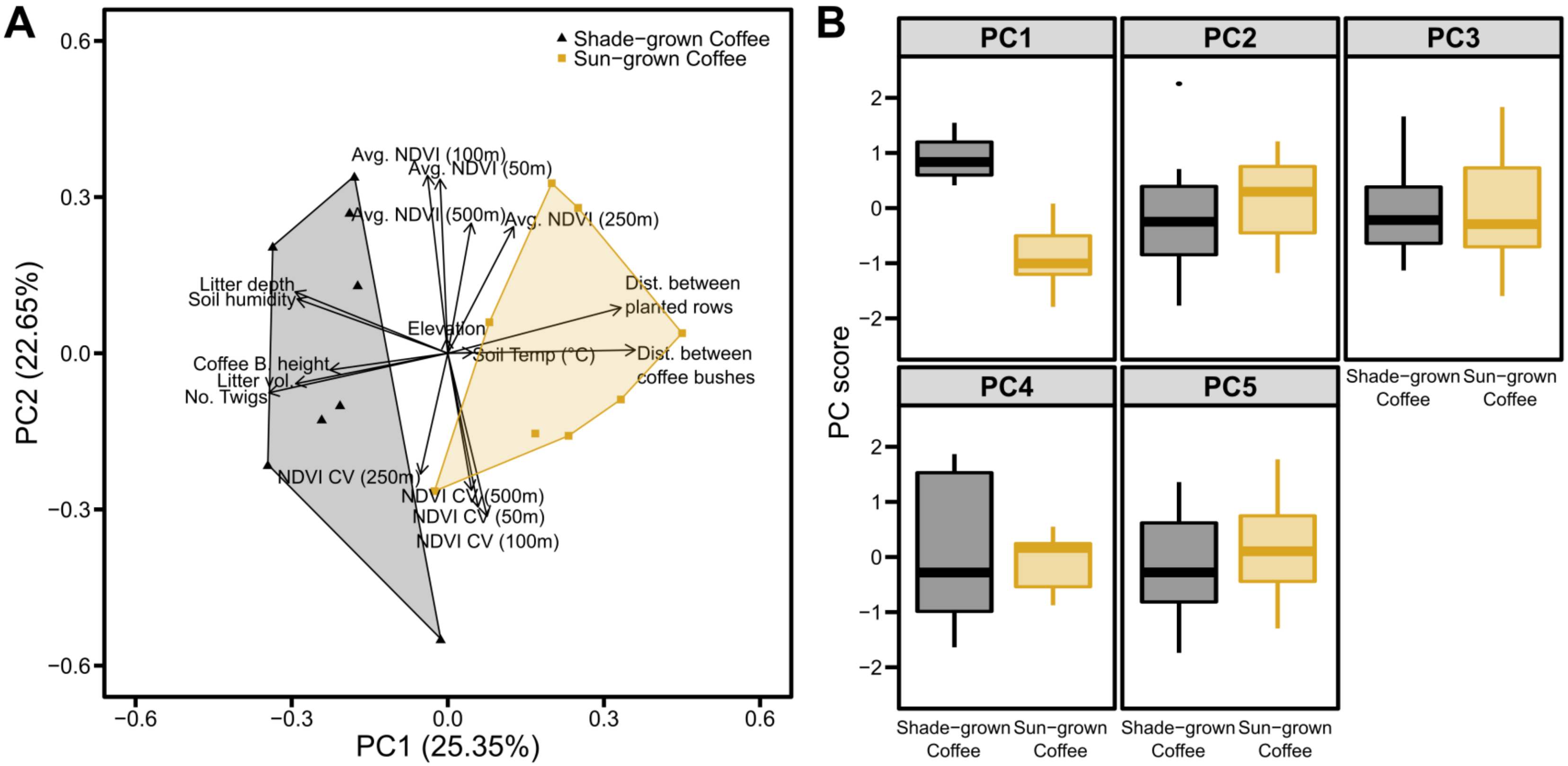
Environmental variable	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**
Local					
Elevation	-0.055	-0.015	0.550	0.333	0.381
Soil Temperature (°C)	-0.233	0.098	**1.002**	0.028	0.390
Soil humidity	0.345	-0.165	0.143	0.222	-0.117
Litter depth	0.781	-0.452	0.013	**0.556**	-0.221
Management					
Coffee bush height	0.609	-0.002	-0.214	-0.309	0.376
Distance between coffee bushes	**-0.880**	0.101	-0.226	0.070	-0.133
Distance between planted rows	-0.820	-0.117	-0.346	0.068	-0.156
Number of twigs	0.845	0.089	0.283	-0.067	-0.057
Litter Volume	0.711	0.098	0.126	-0.330	-0.379
Landscape					
Average NDVI					
50m radius	-0.025	**-0.759**	-0.294	0.090	0.341
100m radius	-0.010	-0.709	-0.072	0.257	0.082
250m radius	-0.400	-0.466	0.521	-0.185	-0.186
500m radius	-0.164	-0.421	0.268	-0.405	0.149
NDVI Coefficient of Variation					-0.158
50m radius	-0.108	0.729	0.345	0.340	0.052
100m radius	-0.088	0.643	0.185	-0.057	**0.432**
250m radius	0.269	0.512	-0.674	0.036	0.119
500m radius	-0.008	0.335	-0.177	0.279	0.341



Supplementary Figure S4.2a. Landsat 8 greenest pixel composite from images between Aug-2015 and Jul-2016 for our sampled region.



Supplementary Figure S4.2b. Boxplots summarizing (A) local scale, (B) management related and (C) landscape scale environmental data for all habitat types. All variables have been standardized (mean of 1 and SD of 0) to allow for comparisons across variables measured in different scales. Different letters represent significant statistical differences ($p < 0.05$) between habitat types. The box encloses the 25–75th percentiles of the values, bold lines represent median and the whiskers extend to 1.5 times the interquartile range.



Supplementary Figure S4.2c. PCA of environmental variables measured in both types of coffee plantations. (A) Biplot depicting the Euclidean distances between the sampled coffee plantations and the eigenvectors of environmental variables measured in the plantations. Different coloured symbols represent different coffee plantations with black triangles and golden squares representing shade-grown and sun-grown coffee plantations, respectively. (B) Boxplots depicting the scores of sampled plantations for each significant PC axis. The box encloses the 25–75th percentiles of the values, bold lines represent median and the whiskers extend to 1.5 times the interquartile range. See Table S2c for contributions to each PC axis for each environmental variable.

APPENDIX S4.3. Supplementary diversity analyses

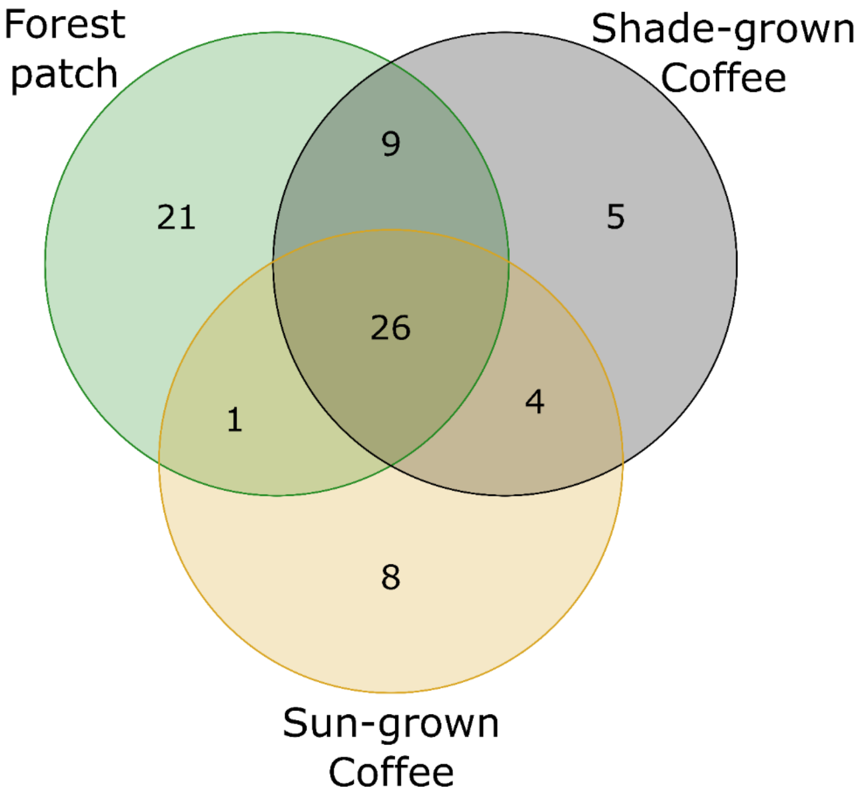
Supplementary Table S4.3a. Summary statistics of PERMANOVA of the effect of environmental variables on taxonomic, phylogenetic, and taxonomic compositional turnover. We used significant environmental PC axes (see Appendix S4.2, Table S4.2b and S4.2c for specific variable contributions) to account for collinearity between variables. Significant variables ($p < 0.05$) are highlighted in bold.

	POOLED			**AUGUST**			**DECEMBER-JANUARY**		
	F	**R^2**	**P**	**F**	**R^2**	**P**	**F**	**R^2**	**P**
Taxonomic dimension									
All habitats									
Environmental PC1	0.371	0.012	0.871	1.191	0.039	0.32	0.262	0.10	0.980
Environmental PC2	**4.316**	**0.139**	**0.001**	**3.843**	**0.125**	**0.005**	**2.616**	**0.100**	**0.026**
Environmental PC3	**2.828**	**0.091**	**0.019**	1.749	0.057	0.133	1.926	0.074	0.101
Environmental PC4	**4.498**	**0.145**	**0.001**	**4.923**	**0.160**	**0.001**	**2.312**	**0.089**	**0.044**
Forest vs Shade-grown									
Environmental PC1	0.364	0.022	0.875	1.129	0.070	0.411	0.083	0.006	0.943
Environmental PC2	1.1619	0.099	0.153	1.174	0.073	0.375	1.108	0.081	0.407
Environmental PC3	1.375	0.084	0.259	0.768	0.048	0.667	1.204	0.088	0.356
Environmental PC4	**2.629**	**0.161**	**0.017**	**2.487**	**0.154**	**0.012**	0.954	0.070	0.504
Forest vs Sun-grown									
Environmental PC1	0.653	0.026	0.672	1.634	0.075	0.163	0.322	0.016	0.857
Environmental PC2	**5.435**	**0.220**	**0.001**	**4.429**	**0.203**	**0.005**	**3.097**	**0.152**	**0.022**
Environmental PC3	**3.165**	**0.128**	**0.019**	1.299	0.059	0.275	**2.969**	**0.146**	**0.026**
Environmental PC4	**3.034**	**0.123**	**0.022**	**2.682**	**0.123**	**0.042**	2.328	0.114	0.062
Shade-grown vs Sun-grown									
Environmental PC1	**12.387**	**0.406**	**< 0.001**	**6.193**	**0.271**	**0.005**	**7.882**	**0.305**	**< 0.001**
Environmental PC2	0.547	0.018	0.710	1.467	0.064	0.246	1.405	0.054	0.277
Environmental PC3	1.357	0.044	0.284	1.440	0.063	0.240	1.765	0.068	0.170

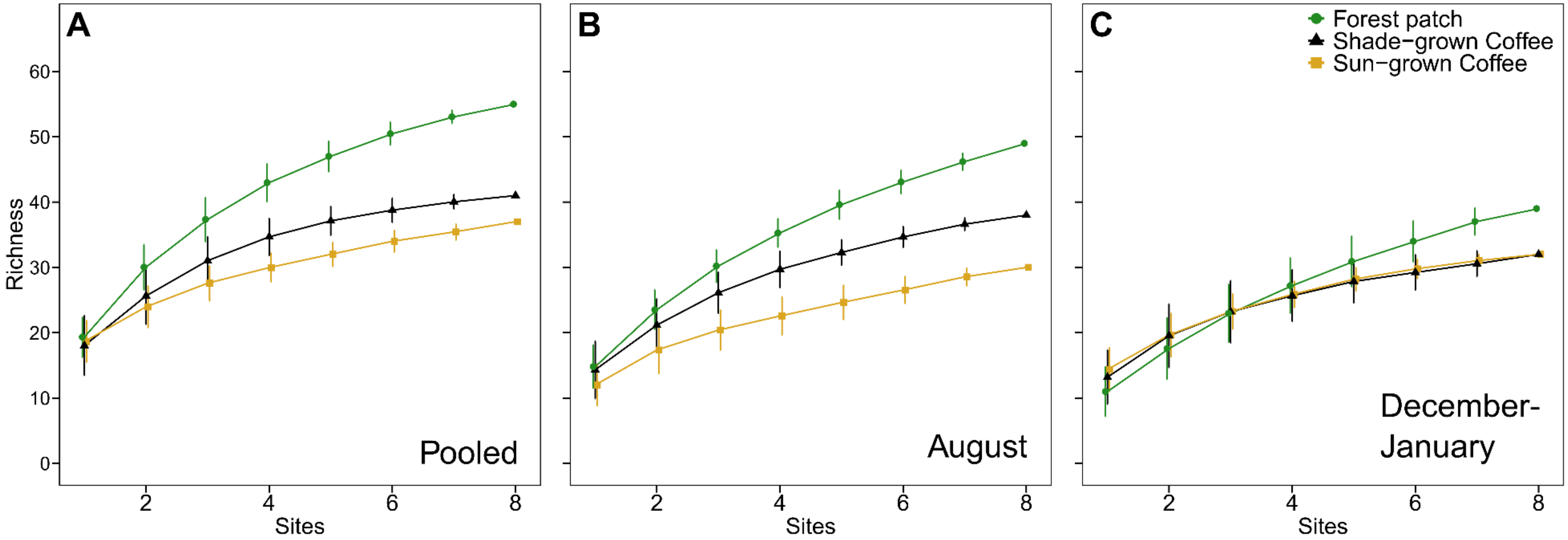
Environmental PC4	**3.062**	**0.100**	**0.044**	1.202	0.053	0.325	**3.729**	**0.144**	**0.011**
Environmental PC5	**3.189**	**0.104**	**0.041**	2.511	0.110	0.085	1.047	0.041	0.436

	POOLED			**AUGUST**			**DECEMBER-JANUARY**		
	F	**R^2**	**P**	**F**	**R^2**	**P**	**F**	**R^2**	**P**
Phylogenetic dimension									
All habitats									
Environmental PC1	0.298	0.009	0.846	0.968	0.030	0.470	1.977	0.068	0.144
Environmental PC2	**7.057**	**0.203**	**< 0.001**	**4.986**	**0.156**	**0.001**	**4.822**	**0.166**	**0.003**
Environmental PC3	**2.719**	**0.078**	**0.040**	1.097	0.034	0.416	1.942	0.067	0.155
Environmental PC4	**5.727**	**0.165**	**< 0.001**	**5.952**	**0.186**	**0.001**	1.270	0.044	0.352
Forest vs Shade-grown									
Environmental PC1	0.388	0.021	0.797	0.749	0.043	0.625	1.985	0.141	0.147
Environmental PC2	**2.479**	**0.135**	**0.040**	0.855	0.049	0.563	0.831	0.059	0.544
Environmental PC3	1.576	0.086	0.222	0.994	0.057	0.487	1.235	0.088	0.381
Environmental PC4	**3.323**	**0.181**	**0.011**	**3.371**	**0.194**	**0.008**	-0.130	-0.009	0.936
Forest vs Sun-grown									
Environmental PC1	0.530	0.017	0.693	2.403	0.091	0.086	1.610	0.07	0.242
Environmental PC2	**9.693**	**0.316**	**< 0.001**	**8.268**	**0.312**	**< 0.001**	**5.193**	**0.226**	**0.005**
Environmental PC3	**3.551**	**0.116**	**0.028**	3.296	0.124	0.031	1.286	0.056	0.346
Environmental PC4	**3.433**	**0.112**	**0.028**	**3.296**	**0.124**	**0.031**	1.286	0.056	0.346
Shade-grown vs Sun-grown									
Environmental PC1	**11.515**	**0.386**	**< 0.001**	**6.633**	**0.262**	**0.003**	**8.946**	**0.366**	**< 0.001**
Environmental PC2	-0.166	-0.006	0.936	1.038	0.041	0.425	1.213	0.05	0.381
Environmental PC3	1.098	0.037	0.408	1.634	0.064	0.232	1.261	0.052	0.357
Environmental PC4	**3.314**	**0.111**	**0.037**	1.471	0.058	0.276	2.335	0.096	0.101
Environmental PC5	**4.052**	**0.136**	**0.019**	**4.566**	**0.180**	**0.012**	0.689	0.028	0.627

	POOLED			**AUGUST**			**DECEMBER-JANUARY**		
	F	**R^2**	**P**	**F**	**R^2**	**P**	**F**	**R^2**	**P**
Functional dimension									
All habitats									
Environmental PC1	0.583	0.019	0.732	1.107	0.037	0.406	0.432	0.019	0.758
Environmental PC2	**4.059**	**0.136**	**0.003**	**3.682**	**0.123**	**0.004**	2.297	0.101	0.091
Environmental PC3	**2.501**	**0.084**	**0.042**	1.971	0.066	0.115	1.253	0.055	0.385
Environmental PC4	**3.793**	**0.127**	**0.005**	**4.254**	**0.142**	**0.002**	-0.187	-0.008	0.928
Forest vs Shade-grown									
Environmental PC1	0.447	0.028	0.805	1.122	0.066	0.448	0.196	0.018	0.823
Environmental PC2	1.641	0.104	0.177	1.287	0.076	0.336	0.497	0.046	0.710
Environmental PC3	1.159	0.074	0.401	0.846	0.050	0.595	0.442	0.041	0.731
Environmental PC4	**2.539**	**0.161**	**0.027**	**2.330**	**0.138**	**0.041**	-0.784	-0.073	0.978
Forest vs Sun-grown									
Environmental PC1	1.016	0.042	0.420	1.836	0.095	0.141	0.379	0.022	0.771
Environmental PC2	**5.274**	**0.217**	**0.001**	**3.561**	**0.184**	**0.008**	2.758	0.157	0.059
Environmental PC3	**3.602**	**0.148**	**0.014**	0.657	0.034	0.675	2.171	0.124	0.124
Environmental PC4	2.342	0.096	0.065	1.874	0.097	0.126	0.744	0.042	0.598
Shade-grown vs Sun-grown									
Environmental PC1	**8.511**	**0.349**	**0.001**	**4.287**	**0.196**	**0.021**	**9.542**	**0.385**	**0.001**
Environmental PC2	0.553	0.023	0.697	0.754	0.035	0.566	2.820	0.114	0.094
Environmental PC3	1.472	0.060	0.281	1.499	0.069	0.254	2.412	0.097	0.134
Environmental PC4	2.159	0.088	0.124	1.880	0.086	0.188	1.773	0.072	0.249
Environmental PC5	1.708	0.070	0.206	**3.446**	**0.158**	**0.042**	-1.757	-0.071	0.996



Supplementary Figure S4.3a. Venn diagram showing ant species occurrence overlap.



Supplementary Figure S4.3b. Species accumulation curves per site per habitat. A) Pooled data, B) August 2015 data, C) December 2015-January 2016 data.

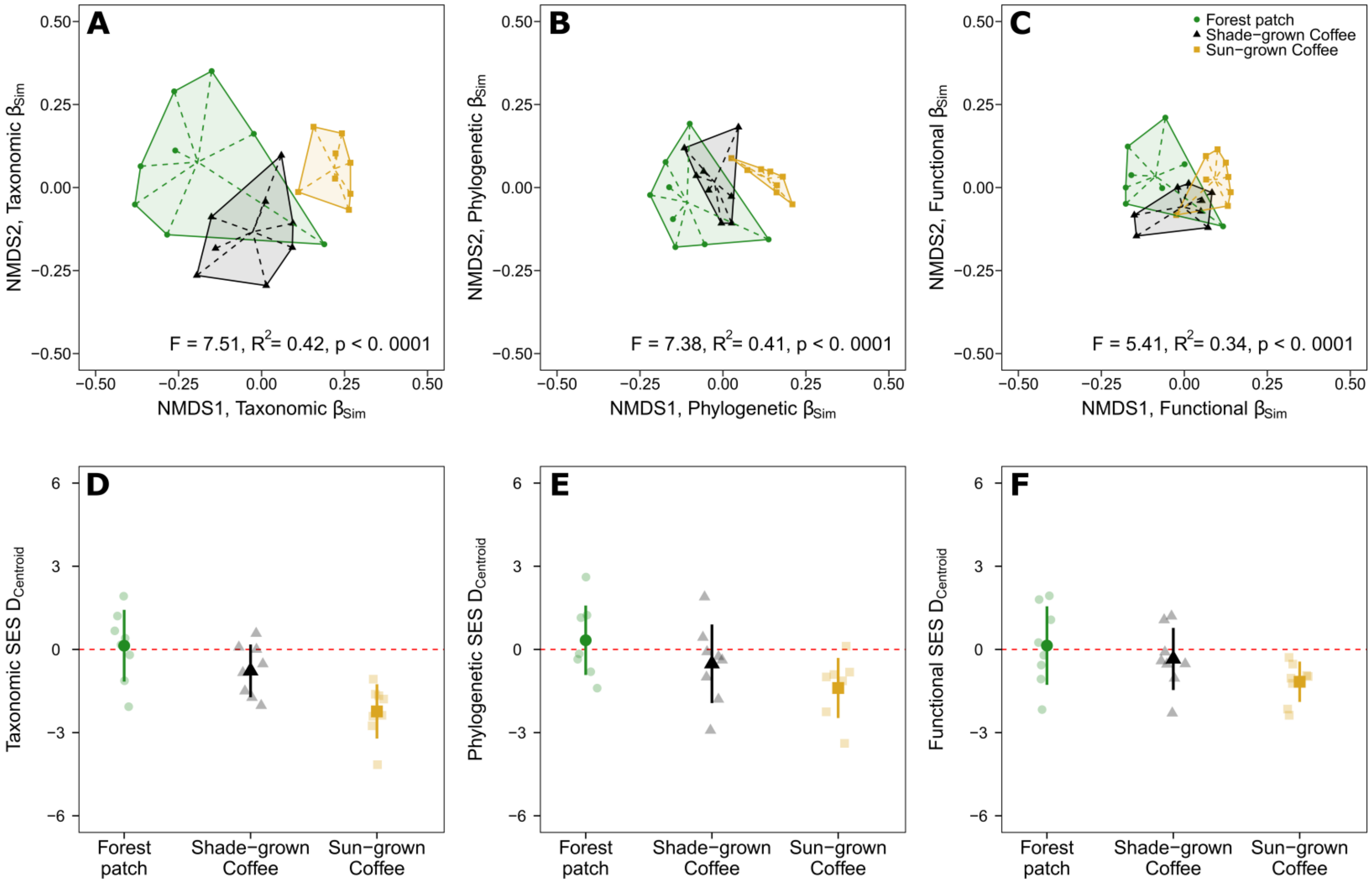
APPENDIX S4.4. Soil-dwelling ant community composition analyses.

Supplementary Table S4.4a. Summary of statistics of the PERMANOVA of the effect of habitat type on taxonomic, phylogenetic, and taxonomic compositional turnover of soil-dwelling ants.

PERMANOVA	**F**	**R^2**	**p-value**
Taxonomic	7.512	0.417	< 0.001
Forest vs Shade-grown	4.081	0.226	0.003
Forest vs Sun-grown	9.897	0.414	< 0.001
Shade-grown vs Sun-grown	10.262	0.423	< 0.001
Phylogenetic	7.381	0.413	< 0.001
Forest vs Shade-grown	3.013	0.177	0.013
Forest vs Sun-grown	10.903	0.438	< 0.001
Shade-grown vs Sun-grown	10.177	0.421	< 0.001
Functional	5.407	0.339	< 0.001
Forest vs Shade-grown	3.272	0.189	0.022
Forest vs Sun-grown	7.573	0.351	< 0.001
Shade-grown vs Sun-grown	5.943	0.298	< 0.001

Supplementary Table S4.4b. Summary of statistics of the PERMDISP analyses of the effect of habitat type on taxonomic, phylogenetic, and taxonomic compositional turnover of soil-dwelling ants.

PERMDISP	**t**	**p-value**
Taxonomic		
Forest	0.292	0.778
Shade-grown	-2.301	0.055
Sun-grown	-6.451	< 0.001
Phylogenetic		
Forest	0.748	0.479
Shade-grown	-1.032	0.336
Sun-grown	-3.631	0.008
Functional		
Forest	0.278	0.789
Shade-grown	-0.869	0.414
Sun-grown	-4.531	0.002



Supplementary Figure S4.4a. Soil-dwelling ant community composition analyses between pairs of local communities in each environment. (A, B, C) NMDS ordination of sites within forest and coffee plantations based on taxonomic, phylogenetic, and functional Simpson's dissimilarity index. (D, E, F) Scatterplot of mean ± SD of standardized effect size of the distance to multivariate space centroid (SES Dcentroid) of each site. Red dashed line represents null expectation.

APPENDIX S4.5. Analysis of ant community trait values

Methods

We calculated the community weighted mean (CWM) for each of the traits we measured. The CWM is the mean trait value weighted by the relative abundance of each species in each site (Swenson, 2014). In the case of continuous trait measurements, CWM is calculated by multiplying the trait value and the relative abundance of each species and then adding these values per site. In the case of binomial measurements (polydomy, polygyny and polymorphism), CWM is the value which is most abundant in one of the categories among all species present in the community (Lavorel et al., 2008).

To test for significant differences between forest patches and management practices, we performed logistic regression analysis (for binomial variables) and ANOVA tests for the rest of traits measured. For binomial variables used the functions *glm* and subsequently the *wald.test* from the package 'aod' (Lesnoff and Lancelot, 2012) to test for overall habitat type effect on the corresponding trait. For continuous traits, we used the function *aov* to assess the overall habitat type effect and *TukeyHSD* to assess specific habitat type differences in trait values. All these analyses were run in R (R Core Team, 2020).

References

Lavorel, S., K. Grigulis, S. McIntyre, N. S. G. Williams, D. Garden, J. Dorrough, S. Berman, F. Quétier, A. Thébault and A. Bonis (2008) Assessing functional diversity in the field - methodology matters! Funct. Ecol. 22:134-147. https://doi.org/10.1111/j.1365-2435.2007.01339.x

Lesnoff, M., Lancelot, R. (2012). aod: Analysis of Overdispersed Data.

R Core Team, 2020. R: A Language and environment for statistical computing.

Swenson, N.G., 2014. Functional and Phylogenetic Ecology in R. New York, NY. https://doi.org/10.1007/978-1-4614-9542-0

Supplementary Table S4.5a. Summary of statistics for CWM analyses.

Trait name	X^2	*p-value*
Polydomy	0.001	0.9
Polygyny*	N/A	N/A
Polymorphism*	N/A	N/A

Trait name	*F*	*p-value*
Diet	2.822	0.082
Body size (Weber's length – WL)	**3.684**	**0.042**
Cephalic index (CI)	2.951	0.074
Maximum Eye Width (MEW)	0.035	0.965
Hind Femur Length (HFL)	2.787	0.084
Pilosity (Hair count)	1.396	0.269
Dominant color	**10.696**	**< 0.001**
Queen Body size (Weber's Length – QWL)	0.832	0.449
Queen Pronotum Width (QPW)	1.576	0.231

*CWM for these traits could not be properly analyzed due to overabundance of zeroes

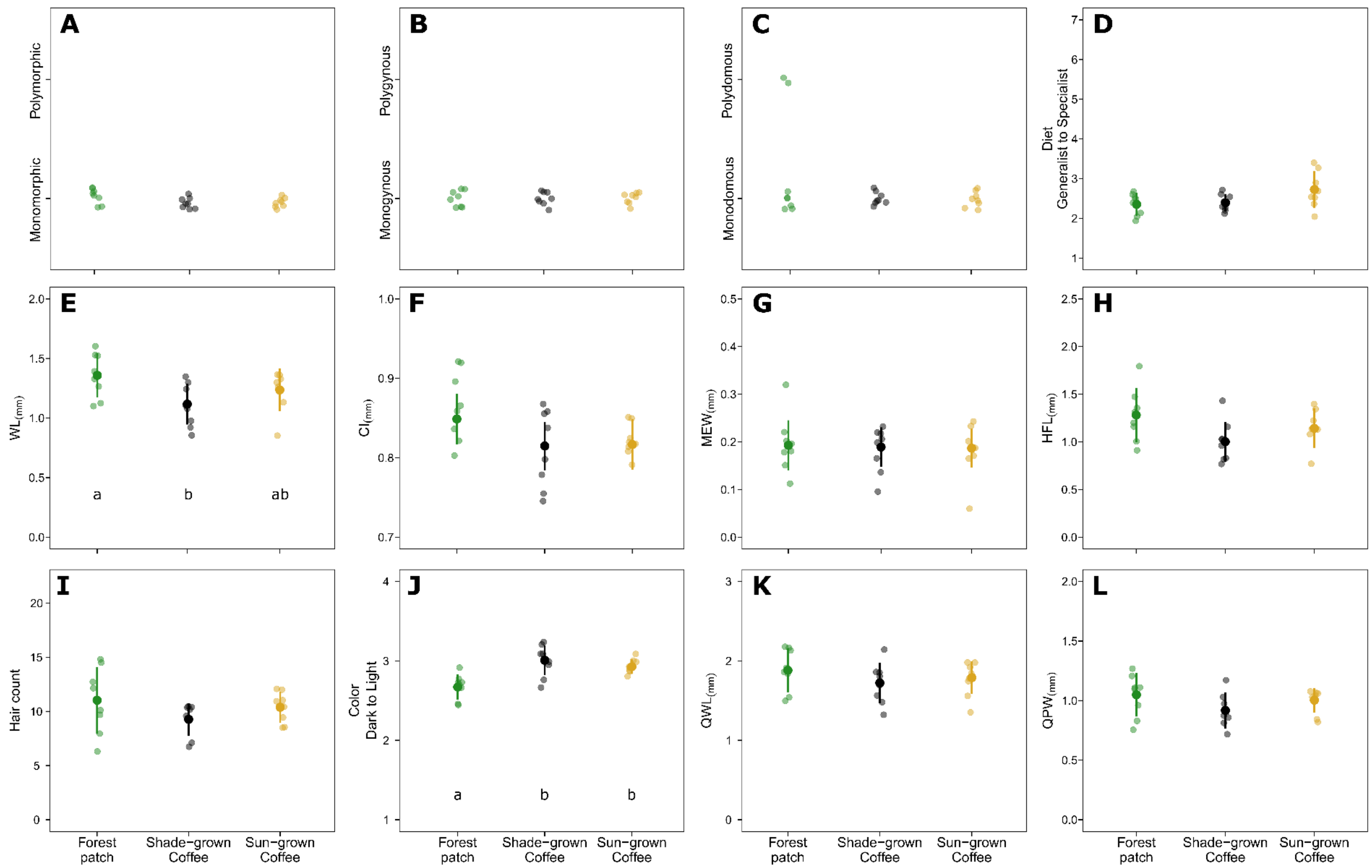


Figure S4.5a. Data spread (light colored circles) of community weighted averages and standard deviation (bold circles and lines) of the different traits measured for this study in the different land use types. **A)** Polydomy, **B)** Polygyny, **C)** Polymorphism, **D)** Diet, **E)** Body size (Weber's length), **F)** Cephalic index (head length divided by head width), **G)** Maximum Eye Width, **H)** Hind Femur Length, **I)** Dominant color, **J)** Number of hairs, **K)** Queen body size (Weber's length), **L)** Queen pronotum width. Different letters denote significant differences after statistical test.

Chapter 5 General discussion

Understanding evolutionary, ecological, and anthropogenic drivers of phenotypic diversity across space and organizational levels has become even more crucial in a rapidly changing world. While many efforts have independently advanced our knowledge of the different factors influencing the observed patterns of diversity, many questions remain. My thesis provides insights into how strong phenotypic integration limits ant lineages to specific climates (chapter 2), how considering intraspecific variation into community-wide studies helps us understand trait adaptations across organizational levels (chapter 3), and the importance of incorporating multiple facets of biodiversity when assessing the effects of anthropogenic change on diversity patterns (chapter 4). Together, the results of my thesis indicate that abiotic factors, such as temperature and precipitation, shape the phenotypic diversity of ants. Moreover, the effects of climate are apparent across organizational levels, indicating that community-wide adaptation can occur at the intraspecific level and may drive lineage-wide phenotypic diversification. However, the anthropogenic reshaping of ecosystems threatens the taxonomical, phylogenetic, and functional homogenization of communities, which can, in turn, threaten the phenotypic diversity of vulnerable (i.e., highly integrated and low trait range) ant lineages.

5.1 Ant phenotypic diversity is supported by climate and habitat diversity

Certain ant lineages are constrained to specific climate zones due to high phenotypic integration (chapter 2). Even after accounting for evolutionary links, strongly integrated ant lineages are found in a narrow range of climates. In contrast, ant lineages that are found in various climates show low phenotypic integration. These weakly integrated ant lineages can live in a variety of climate zones, perhaps because of certain species within that lineage that developed worker polymorphism (i.e., colony-level plasticity), which would enable them to live in diverse climates. Indeed, warm and arid regions of the world have promoted the emergence of polymorphic ant colonies (La Richelière et al. 2022). Evidence suggests that this colony polymorphism has persisted over evolutionary time in multiple lineages, even after speciation events (Kay et al. 2022). Consequently, it is possible that the evolution of worker polymorphism allowed ant lineages to explore different areas of trait space and may be key in explaining the ecological success of certain ant lineages (Rajakumar et al. 2012, Powell et al. 2020). However, further studies are needed to better understand the relationship between phenotypic integration and worker polymorphism in ants.

The maintenance of ant phenotypic variation depends heavily on the variety of habitats found on Earth. However, climate change and land use are impacting the distribution and diversity of biomes, which threatens to reduce opportunities for maintaining phenotypic diversity. This could lead to phenotypic homogenization, especially for strongly phenotypically integrated and/or less morphologically diverse lineages (Chapters 2 and 4). Previous works have also found a potential reduction of phenotypic diversity due to the increased selective pressures of intensified management strategies (Armbrecht et al. 2005, Philpott et al. 2008, Urrutia-Escobar and Armbrecht 2013) and land conversion in general (Sales et al. 2020). Furthermore, there is evidence that the ecological strategies of species are disappearing and will continue to decline in light of climate change (Cooke et al. 2019). Therefore, it is crucial to study the range and integration of non-morphological traits to gain deeper insights into the mechanisms underlying phenotypic diversity. In addition, understanding how lineages may react to these environmental changes can inform prediction models and have important applications to guide conservation measures.

5.2 Ant communities are structured by adaptation to environmental conditions

Ant communities in extreme environments (e.g., cold and dry) are structured by community-wide trait adaptation rather than plasticity (chapter 3). This means that the entire community of ants is adapted to the harsh environmental conditions, rather than community-level trait plasticity in response to changing conditions. In other words, ant species living in extreme environments have evolved to possess traits that enable them to survive and thrive in those environments. Previous works have indicated that these may go beyond morphological traits and may include behavioral adaptations such as changes in foraging patterns (Retana and Cerdá 2000), as well as physiological adaptations such as thermal and drought tolerance (Cerda et al. 1998, Baudier et al. 2015). Chapter 3 suggests that the specific morphological traits that are important for community-wide adaptation are eye size and antennae length. This finding is significant because it suggests that individual ants have evolved to adapt to extreme environments and that this is reflected across organizational levels (i.e., worker, colony, and species). Other studies, for example, have found that ants become larger and darker in colder environments (Bishop et al.

2016) or have shorter antennae in complex environments (Nooten et al. 2019). Notably, these studies have been carried out under different conditions (natural vs anthropogenic) and in different regions of the world, which suggests that other factors such as biogeographical history and resource availability can also influence the observed trait composition of ant communities.

Chapter 4 indicates that ant communities are susceptible to changes in environmental conditions, indicating that species vulnerability is also reflected at the community level. Habitat openness has been suggested as a major mediator of diversity change in biological communities (Andersen 2019). In ants, this can be seen with certain genera preferring to establish their colonies in open environments, where they are more productive (Vieira-Neto et al. 2016). Consequently, a drastic change in habitat openness can affect the ecological services provided by ants given that the diversity of the ant community is also affected (Wielgoss et al. 2014). An important thing to note, however, is that habitat openness needs to be considered from the perspective of the ants. From their perspective, this may mean the amount and composition of leaf litter and the presence of herbaceous plants and shrubbery. Indeed, in Chapter 4 we can observe that local conditions of shade-grown farms had denser and more diverse vegetation (i.e., coffee shrubs) and deeper and more humid leaf litter than sun-grown plantations and that this follows taxonomically, phylogenetically, and functionally distinct ant communities.

5.3 The influence of anthropogenic environmental change in ant diversity

Climate change threatens to reduce opportunities to maintain ant diversity. Collectively, my thesis joins previous work in suggesting the importance of understanding the relationship between ant diversity and climate (e.g., Sanders et al. 2007b, Dunn et al. 2009, Arnan et al. 2014, Fowler et al. 2014, Gibb et al. 2015, Penick et al. 2017). My thesis then goes a step further in examining the effects on lineage-wide phenotypic diversity (chapter 2), across space while considering within-community trait variation (chapter 3), and at the community level under contrasting environments following anthropogenic disturbance (chapter 4). These results demonstrate that lineages and communities are susceptible to anthropogenic disturbance and that they are at risk of homogenization. Other studies have extended these to other taxa, including beetles (Rivera et al. 2023), birds (Devictor et al. 2008), mammals (Fraser et al. 2022), and plants (Olden 2006). Therefore, increasing the understanding of key mechanisms underlying the influence of anthropogenic disturbance on biodiversity loss is of the utmost importance to discuss appropriate mitigation strategies that target not only the identity of species but also their evolutionary history and their potential roles in the ecosystem.

Anthropogenic environmental changes, such as deforestation, urbanization, and agricultural intensification, can severely impact ant diversity. Since ants play a crucial role in maintaining ecosystem health and providing ecosystem services (Del Toro et al. 2012), the loss of ant diversity can have far-reaching impacts. The presence of diverse ant communities in plantations can provide important ecosystem services, such as pest control and soil nutrient cycling. Ants can help to control pest populations by preying on insect pests, which can reduce the need for synthetic pesticides (Aristizábal and Metzger 2019). Additionally, ants can also help to improve soil health by breaking down organic matter and increasing soil nutrient availability (Del Toro et al. 2015). Therefore, conserving and promoting ant diversity in plantations through sustainable practices can have important environmental and economic benefits.

5.4 Future areas of research

Based on the results of my thesis work, there are several potential areas for future research on ant phenotypic diversity. Specifically, my work suggests that climate shapes the phenotypic diversity of ant lineages and communities. One avenue would be to further investigate the role of phenotypic plasticity in the expansion of ant genera into deserts and other extreme environments. In the case of ants, phenotypic plasticity can be seen as the ability of a colony to present worker polymorphism, which has been suggested to be an important factor in the ecological success of ants (Wills et al. 2018). Thus, it would be interesting to see whether the degree of lineage-wide phenotypic integration is associated with polymorphic vs monomorphic genera, and how this may be related to the success of certain ant lineages. Another area of research is to explore the relationship between phenotypic integration and its effect on the diversification of ant lineages and castes, including whether all ant lineages have the same rate of phenotypic diversification and how strong trait integration may mediate caste and subcaste differentiation in certain lineages.

Though throughout my thesis I explored ant phenotypic diversity across space and climatic conditions, an interesting aspect that was not considered is how trait distributions and phenotypic diversity would vary when considering non-morphological traits and biotic factors. Firstly, ant ecological strategies consider life history traits such as foraging strategy and habitat preference (Gibb et al. 2023). Though Chapter 2 offers an important first step into understanding the covariance of morphological traits across lineages and across castes, future work would benefit from including other colony-level traits (e.g., worker number, polymorphism, foraging strategy). Exploring the covariance among these traits may lead to a proper framework that indicates the most important and easily measured traits associated with ant ecological strategies. Secondly, considering that competition has been highlighted as a hallmark in determining ant distribution (Hölldobler and Wilson 1990, Cerdá et al. 2013), future work should consider this aspect when seeking to expand our understanding of the drivers of phenotypic diversity in ants. Given that ants exist in multispecies communities, an understanding of how biotic factors such as intra- and interspecific competition may drive phenotypic diversification and how this interacts with strongly integrated lineages would be an interesting avenue to explore in future work.

5.5 Conclusion

Overall, my thesis contributes important points to our understanding of the drivers of the phenotypic diversity of ants. First, climate diversity plays an important role in maintaining ant diversity by supporting highly integrated phenotypes, which are limited to a narrow range of climate zones and are therefore vulnerable to climate homogenization. Second, considering the social complexity of ants (worker vs colony-level traits) is important when investigating trait variation across space because selection pressures may act differently at the worker and colony levels. Indeed, because of their eusocial nature, ants have phenotypic flexibility that may enable many species to adapt to changing environmental conditions in a way that non-social organisms are unable to (Parr and Bishop 2022). Finally, anthropogenic disturbance influences the identity, evolutionary history, and potential roles of species that occur within a community. Considering this, my work has important implications for our understanding of how phenotypically complex organisms respond to abiotic factors and provides some insights into how climate change and anthropogenic disturbance may cause a decline in phenotypic diversity.

From a conservation perspective, my work adds to decades of research looking into the response of ants to climate change. The results of my thesis indicate that strongly phenotypically integrated lineages and less diverse communities are the most vulnerable to climate change. Ensuring the maintenance of climate and habitat diversity will play an important role in supporting the vast diversity of ant phenotypes. Furthermore, studies looking into multiple facets of biodiversity are better suited to inform conservation efforts. Considering the phylogenetic and functional aspects of diversity can be useful for designing conservation strategies that target vulnerable lineages and phenotypes that play important roles in ecosystems, such as specialist predators. By taking a holistic approach to biodiversity assessment, we can better inform policymakers and stakeholders not only for the conservation of ant diversity but for other taxa as well that benefit from the myriad of ecosystem processes in which ants are involved.

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