Ant nests differentially affect soil chemistry across elevational gradients

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Abstract
Ants alter soil moisture and nutrient distributions during foraging and nest construction. Here, we investigated how the effects of ants on soil vary with elevation. We compared moisture, carbon, and nitrogen levels in soil samples taken both within nests and nearby the nests (control) of two subterranean ant species. Using a paired design, we sampled 17 sites along elevation gradients in two California mountain ranges (Formica francoeuri in the San Jacinto mountains and Formica sibylla in the Sierra Nevada). We observed an interaction between soil carbon and nitrogen composition and elevation in each mountain range. At lower elevations, nest soil had lower amounts of carbon and nitrogen than control soil, but at higher elevations, nest soil had higher amounts of carbon and nitrogen than control soil. However, our sampling method may only breach the interior of ant nests in some environments. The nest soil moisture did not show any elevational patterns in either mountain range. Ants likely modulate soil properties differently across environmental gradients, but testing this effect must account for variable nest architecture and other climate and landscape differences across diverse habitats.

Keywords Soil nutrients · Elevational gradient · Formica · Ant nest · Ecosystem engineer · Landscape-scale

Introduction
Ants are characterized as ecosystem engineers (Jones et al. 1994; Folgarait 1998). Subterranean ant species alter soil hydrology and nutrients by excavating underground nests, thus modulating flows of limiting resources and increasing the complexity of the subterranean habitat (Crist and Wiens 1996). They increase soil drainage and aeration through the formation of underground tunnels (Alvarado et al. 1981) and incorporate nutrients such as carbon and nitrogen into soil through food storage (Friese and Allen 1993), aphid cultivation (Folgarait 1998), and the accumulation of feces and corpses (Gay 1993). For example, the effect of harvester ants on vegetation is likely influenced more by their creation of soil nests than by their role as seed harvesters (Wilby et al. 2001). Indeed, the local effects of active ant colonies on soils are well known (Nkem et al. 2000; Moutinho et al. 2003; Sankovitz and Breed 2019).

Although soil bioturbation by ants is a fine-scale disturbance, it induces landscape-level changes by allowing infiltration (Eldridge 1994; Whitford and Others 2000) and redistribution of nutrients (Nkem et al. 2000). The functional significance of these structures is likely to vary, and the same type of soil disturbance may produce variable effects in different habitats (Steinberger and Whitford 1984; Whitford and DiMarco 1995; Snyder et al. 2002). Few studies, however, have considered the effects of ant-created structures across different habitats (though see Whitford and DiMarco 1995; Snyder et al. 2002; James et al. 2008; Farji-Brener and Werenkraut 2017), even though landscape-scale spatial variability in climate, disturbance, and resource distribution is an essential determinant of many ecosystem processes (Peters and Havstad 2006).

Here, we focus on scaling up from local to landscape-scale soil effects in ant species that span environmental gradients. We investigated how two ant species (Formica francoeuri (Bolton, 1995) and Formica sibylla (Wheeler, 1913)) affect both soil moisture and soil chemical properties in mountain ranges of California. Both species build inconspicuous subterranean nests. We conducted this study across two elevational gradients of ~1000 m in the Sierra Nevada and San Jacinto mountain ranges.
The aim of the present study was to investigate two questions: Do soil moisture and levels of carbon and nitrogen differ consistently between ant nest soils compared with adjacent non-nest soils, irrespective of ant species or habitat? and (ii) change predictably with the abiotic environment along an elevational gradient? Per our knowledge of ants as ecosystem engineers, we predicted that soil properties would be significantly different in nests compared to control soil. In particular, we expected to observe an enrichment of carbon and nitrogen in nest relative to control samples. Further, since many factors that influence soil quality and ant behavior—such as temperature (Sankovitz and Purcell 2021), precipitation (Krushelnicki et al. 2005), soil type (Kumar and O’Donnell 2009), and vegetation (Wagner and Fleur Nicklen 2010)—vary with elevation, we predicted that the impact of ants on soil properties would vary with elevation. However, we did not have an a priori expectation about the orientation of this relationship.

Materials and methods

Study area and sampling methods

We conducted this study in June–July 2018 within the San Jacinto Mountains and the Sierra Nevada, California, USA. We sampled soil and workers from the F. francoeuri colonies across eight sites along two transects (on north- and south-facing slopes) in the San Jacinto Mountains (spanning 371–1370 m) and the F. sibylla colonies across nine sites along two transects (on east- and west-facing slopes) in the Sierra Nevada (spanning 1776–2350 m) (Table S1). These two species are common within their respective mountain ranges, but they are not found in sympatry. Since many Formica species look similar, confirming species identification based solely on morphology can be challenging. Therefore, we sequenced 1–2 ant workers per colony for genetic species identification (see ‘Species Confirmation’ section in supplementary materials for detailed methods and results).

At each nest, we sampled soil from both the nest itself and a control area approximately 10 m away, in a direction chosen with a random number generator. We took samples from a depth of approximately 10 cm (Nkem et al. 2000; Wagner et al. 2004) using a trowel (samples were approximately 10×10 cm², spanning 10–20 cm deep). Additionally, to investigate how ant nest architecture may affect soil properties in contrasting environments, we revisited one low and one high elevation site in the San Jacinto Mountains and took soil cores 35 cm deep (three nest cores and one control core per site). We sampled soil from four depths in each core.

Measurement of soil moisture and chemical properties

We sieved (2 mm) each soil sample to exclude rocks and sticks and divided the samples into halves. Using one half, we immediately measured the wet weight and then dried the samples in an oven at 105 °C for 24 h. To determine moisture content, we then measured dry weight, calculated the difference between the two weights, and divided the difference by the dry weight to standardize by volume. We dried the other half sample at 55 °C overnight, then thoroughly ground and passed it through a 100-mesh sieve. We homogenized the soil and packaged ~ 80 mg of each sample into a tin capsule. Samples were analyzed for total carbon and nitrogen content at the UCR Environmental Sciences Research Laboratory using a Flash EA1112 elemental analyzer.

Statistical analyses

We performed all statistical analyses in R (V 5.3.5, The R Foundation for Statistical Computing). We used the lmer function from the lme4 package (Bates et al. 2014) and the lmerTest function from the lmerTest package (Kuznetsova et al. 2017) to build a linear mixed-effects model for each species in order to compare the nest and control soil across elevation. We built two separate models because each ant species is found in only one mountain range, so we cannot distinguish between range-specific and species-specific differences in our analysis. In these models, soil C, N, and moisture were response variables, sample location (nest or control), elevation, and the interaction between the two were fixed effects, and nest ID and transect were random effects. To examine if there was an effect of transect, we used a likelihood ratio test to compare models with and without transect as a random effect. Additionally, we ran linear mixed effect models to examine whether baseline soil properties vary with elevation in the control samples from each mountain range. In these models, soil C, N, and moisture were response variables, elevation was a fixed effect, and the interaction between the two were fixed effects, and nest ID and transect were random effects. To examine if there was an effect of transect, we used a likelihood ratio test to compare models with and without transect as a random effect. Additionally, we ran linear mixed effect models to examine whether baseline soil properties vary with elevation in the control samples from each mountain range. In these models, soil C, N, and moisture were response variables, elevation was a fixed effect, and transect was a random effect. To examine the variance of carbon and nitrogen at various depths throughout the nest, we used linear models to analyze the results of the 35 cm nest soil cores. In these models, soil sample depth was the independent variable, and the percentage of carbon or nitrogen was the response variable.
Results and discussion

The difference in soil carbon and nitrogen content between ant nests and control sites varied with elevation. The extent to which control soil was enriched with nitrogen was significantly positively correlated with elevation (and the climatic factors that covary with elevation (Table S2)) across both mountain ranges, and the extent to which control soil was enriched with carbon was significantly positively correlated with elevation in the Sierra Nevada (Table S3). At higher elevations, nest soil had higher amounts of carbon and nitrogen (Fig. 1; Table 1) than adjacent control soil samples. This pattern was consistent with our expectations. However, at lower elevations, nest soil tended to have reduced carbon and nitrogen relative to controls (Table 1), which seemed counter-intuitive. The unexpected pattern of nutrient depletion in low elevation ant nests led us to consider possible limitations of studies investigating soil properties in ant nests. While moisture of control soil significantly increased with elevation in the Sierra Nevada, there was no significant pattern along the elevational gradient of the San Jacinto Mountains (Table S3). We observed no significant pattern of soil moisture in ant nests compared with controls (Table 1). Here, we describe and interpret the patterns that we found in our study, and then we consider potential sources of noise in our sampling approach and suggest steps that could be taken to reduce variance not associated with the biological research questions in future studies.

Intriguingly, data from both ranges showed a trend of low elevation nests containing soil depleted in carbon and nitrogen compared to control soils, whereas high elevation nest soils were enriched in carbon and nitrogen compared to control soils. The dotted line represents the baseline amount of carbon or nitrogen in the (control) soil.
to control soils. While these consistent trends emerged, the magnitude of ant nest effects on soil varied between the mountain ranges and transects within range. The relative increase or decrease in carbon and nitrogen levels in ant nest soil compared to control soil was up to an order of magnitude greater in the *F. sibylla* nests in the Sierra Nevada compared to the *F. francoeuri* nests in the San Jacinto mountains. Thus, our results highlight the complexity of generalizing the effects of ant nests on soil properties across elevations and landscapes that may vary in water drainage, soil type, and climate.

We were unsurprised to find higher soil carbon and nitrogen in nest soil compared with control soil in many sites. This result is consistent with studies that have found that *Formica* nests contain higher amounts of nutrients and organic matter (Kristiansen and Amelung 2001; Drager et al. 2016), which suggests a large community of soil microbes in nest soils that would encourage greater rates of decomposition and nitrogen mineralization (as has been found in harvester ants (Wagner and Jones 2006)). We also expected differences in the effects of nests across elevation gradients, as landscape-level processes affect the distribution of soil nutrients. Our results align with studies that have found a differential effect of ants on soil processes and function across various climates and land-use regimes (Folgarait 1998).

The result that nest soils showed nutrient depletion compared to control soil in many lower-elevation sites was more surprising. We identify three alternative, biologically relevant hypotheses that may explain this counter-intuitive finding. The pattern could result from soil texture and permeability; generally, more sandy soil is nutrient-poor because nutrients tend to leach through (e.g., Ge et al. 2019). Observationally, higher-elevation sites appeared to have more compact soil, which may limit the depth of nests and resulting nutrient accumulation. Conversely, ants may excavate deeper in less-compact soil, bringing vegetation and prey items well below the soil surface and bringing nutrient-poor soil to the surface. Our complementary research revealed that ant nest depth depends on surface temperature in laboratory-based groups of *Formica podzolica* workers (Sankovitz and Purcell 2021). Hence, nest architecture may vary consistently with elevation, causing standard soil sampling approaches, like the one used here, to yield samples from different portions of nests at different points along elevation gradients. To investigate the possibility that our standardized sampling approach systematically collected soil from different portions of nests at different points along elevation gradients. To investigate the possibility that our standardized sampling approach systematically collected soil from different portions of nests across sites, we revisited one low and one high elevation site in the San Jacinto Mountains and took soil cores 35 cm deep. However, we found no correlation of carbon or nitrogen with soil depth in these preliminary samples (Table S4).

Several issues with our sampling protocol could yield significant differences in soil metrics that are not mediated by biologically interesting effects of ant nests on soil and should be adjusted in future studies. First, even if

### Table 1 Linear mixed-effects model results

| Mountain range          | Soil property | Variable                          | DF   | F      | P      |
|-------------------------|---------------|-----------------------------------|------|--------|--------|
| San Jacinto             | C             | Treatment or control              | 1,30 | 8.5180 | 0.006608** |
|                         |               | Elevation (m)                     | 1,29.277 | 0.2959 | 0.590603 |
|                         |               | Treatment or control × elevation (m) | 1,30 | 11.0332 | 0.002362** |
|                         | N             | Treatment or control              | 1,30 | 5.8779 | 0.021567* |
|                         |               | Elevation (m)                     | 1,30 | 0.7561 | 0.391446 |
|                         |               | Treatment or control × elevation (m) | 1,30 | 10.3889 | 0.003051** |
|                         | Moisture      | Treatment or control              | 1,30.002 | 0.2455 | 0.6239 |
|                         |               | Elevation (m)                     | 1,29.999 | 0.3651 | 0.5502 |
|                         |               | Treatment or control × elevation (m) | 1,30.002 | 0.2482 | 0.6220 |
| Sierra Nevada           | C             | Treatment or control              | 1,39 | 6.7280 | 0.01330* |
|                         |               | Elevation (m)                     | 1,39 | 5.3051 | 0.02668* |
|                         |               | Treatment or control × elevation (m) | 1,39 | 9.2211 | 0.00425** |
|                         | N             | Treatment or control              | 1,38.999 | 8.5375 | 0.005761** |
|                         |               | Elevation (m)                     | 1,1.1612 | 1.9928 | 0.320231 |
|                         |               | Treatment or control × elevation (m) | 1,38.999 | 11.3626 | 0.001701** |
|                         | Moisture      | Treatment or control              | 1,39 | 0.0466 | 0.830159 |
|                         |               | Elevation (m)                     | 1,38.387 | 9.3748 | 0.004005** |
|                         |               | Treatment or control × elevation (m) | 1,39 | 0.0648 | 0.800466 |

The extent to which nest soil was enriched with carbon and nitrogen was significantly correlated with elevation across both mountain ranges. Significance at level 0.001 is denoted with ‘**’; and significance at level 0.01 is denoted with ‘*’.
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throughout homogenized, the quantity of soil used by the elemental analyzer is minuscule—mere grams—making obtaining a representative sample difficult. In this study, we took measurements from a single soil sample within the nest and a second one near the nest (nest and control sample, respectively). Comparing multiple nest and control samples for each nest could provide a more representative measure. Second, ant nests complicate this process further because the bioturbation and addition of organic matter by ants, in addition to variation in nest architecture, create a plot of soil that is highly variable in all three dimensions. We took samples from a standard 10 cm below the soil surface, following established protocols (Nkem et al. 2000; Wagner et al. 2004), but our concurrent study indicated that nest depth could strongly covary with elevation and temperature (Sankovitz and Purcell 2021). Hence, we may have breached the nest in some samples and not others, with a bias associated with the average temperature of each locality. This sampling approach might be accurate for ants that excavate superficial nests, like Argentine ants, but both sampling soil cores and investigating the depth of the highest nest chambers may be necessary to ensure that equivalent samples are compared in ant species that dig deeper nests. Due to these potential limitations, the results presented here should be considered intriguing patterns that hint at ecologically significant processes at play but should be investigated further with sampling encompassing a broader spectrum of possible nest architecture.

Much research has focused on the effects of ants on ecosystem processes within single populations (De Bruyn and Conacher 1990; MacMahon et al. 2000; Decaëns et al. 2002; Folgarait et al. 2002), but few studies have examined how these effects vary across environmental gradients. Both our original samples and the follow-up samples taken using a soil core contained significant variability, demonstrating the difficulty of taking a representative soil sample inside ant nests. Despite our efforts to homogenize soil samples, analyzing mere grams of soil from an entire nest probably does not capture a representative profile of nest soil. We recommend that future research on ant soil interactions sample soil from multiple locations within an ant nest, both parallel and perpendicular to the soil surface. Overall, our results suggest that the magnitude of ant nest effects on nutrients is likely influenced by factors that vary with elevation, like climate and soil type. Future research on ant ecosystem engineering should work to generalize how ants affect soil properties in distinct geological and climatic zones.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s00040-022-00869-1.

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**Author contributions** MS and JP contributed to the study conception and design. MS performed material preparation, data collection, and analysis. MS wrote the first draft of the manuscript, and both authors commented on previous versions of the manuscript. Both authors read and approved the final manuscript.

**Declarations**

**Conflict of interest** The authors have no conflicts of interest.

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