

Effects of *Formica podzolica* ant colonies on soil moisture, nitrogen, and plant communities near nests

MADISON A. SANKOVITZ,  MICHAEL D. BREED

and HELEN F. MCCREERY Department of Ecology and Evolutionary Biology, University of Colorado at Boulder, Boulder, Colorado, U.S.A.

Abstract. 1. Ants are widely regarded as ‘ecosystem engineers’ because their nest construction and contributions to nutrient cycling change the biological, chemical, and physical properties of the soil around their nests. Despite increasing attention to ant manipulation of soil ecosystems, the extent to which many common species influence soil properties, as well as nutrient uptake and community composition of plants near nests, is still unknown.

2. This study tested hypotheses that activities of a common subalpine ant, *Formica podzolica*, alter soil moisture and pH, redistribute nitrogen around nests, and affect plant species abundance and ground cover.

3. A combination of field sampling techniques showed that distance from a nest had a positive relationship with soil moisture and a negative relationship with plant abundance next to and downhill from nests. Slope aspect also affected plant communities, with downhill transects having higher plant cover and above-ground biomass than uphill transects. A stable isotope analysis did not reveal that plants near nests had enriched ^{15}N , but there were substantial differences in ^{15}N among sites.

4. Overall, this study uncovers significant impacts of *F. podzolica* on the subalpine microhabitats directly surrounding their nests.

Key words. Ant, *Formica*, ecosystem engineer, plant diversity, stable isotope analysis.

Introduction

Social insects dramatically alter the ecosystems in which they live (Jones *et al.*, 1994). Ants do this by modifying the biological, chemical, and physical properties of the soil in and around their nests through nest construction, foraging, and other contributions to nutrient cycling (Folgarait, 1998; Hillebrand *et al.*, 2007; Frouz & Jilková, 2008; Domisch *et al.*, 2009; Pinkalski *et al.*, 2017; Rosumek, 2017). Understanding fine-scale interactions between ant nests and the communities that surround them can provide critical links for integrating local drivers of change with community and ecosystem processes.

Soil alterations by ants occur as a result of a variety of colony activities carried out by workers (Souza *et al.*, 2015; Bujan *et al.*, 2016; Farji-Brener & Werenkraut, 2017). Foraging, in which ants gather vegetation and prey items, brings an influx of nutrients to the nest area. Ants also discard corpses outside their nests (Beattie, 1989; Sun & Zhou, 2013). Sagers

et al. (2000) found, through a stable isotope analysis, that ants make substantial contributions of carbon and nitrogen to their surrounding environment by way of deposited debris. Nitrogen secreted in the form of uric acid enriches nest soil, often leading to a shift in soil pH towards neutral (Frouz & Jilková, 2008). Arboreal ants (*Azteca*) have also been shown to enrich the soil beneath their nests, demonstrating the generality and widespread influence of ants on soil ecosystems (Clay *et al.*, 2013). These inputs can result in a greater buffering capacity in acidic soils. Changes in soil moisture often occur as a result of bioturbation, which can lead to more porous soil around nests (Frouz & Jilková, 2008). These alterations to the biological, chemical, and physical environments around nests favour microorganisms and, thus, decomposition processes, creating more available nutrients for plants (Folgarait, 1998; Wagner *et al.*, 2004; Sousa-Souto *et al.*, 2012). Hence, nest-building and foraging activities have both long- and short-term effects on soil through structural alterations, nutrient accumulation and release, and possible enhancements of soil organic matter (Nkem *et al.*, 2000).

Soil quality is a critical factor in plant community composition, so the influence of ant nests on vegetation has interested researchers for decades. Lesica & Kanno (1998) showed

Correspondence: Madison A. Sankovitz, Department of Ecology and Evolutionary Biology, University of Colorado at Boulder, Boulder, CO 80309, USA. E-mail: madisonsankovitz@gmail.com

that ant mounds can offer a warm, aerated, nutrient-enriched environment for plants. Hence, nests create habitat for numerous species of plants that otherwise could not propagate in cold, waterlogged soil. In addition to soil structural changes, ants can influence plants by enhancing nutrients in soil. Wood ants [*Formica rufa* (Linnaeus, 1761)] increase nitrogen cycling in boreal forests of eastern Finland through their foraging (Finér *et al.*, 2012). Ants directly modify plant communities by moving seeds (myrmecochory) (Vander Wall *et al.*, 2005; Zelikova *et al.*, 2008) and by pruning leaves and plant stems in areas around their nests (Davidson *et al.*, 1988). Iakovlev *et al.* (2017) demonstrated that trophic position of a foraging generalist ant can shift seasonally with changing resource availability, meaning that effects of ants on soil nutrients may shift seasonally. Mutualisms between ants and other insects provide similar examples of how nutrients, ant foraging behaviour, and plant community composition are interdependent (Hernandez *et al.*, 2017).

Because ants may closely link to the ecological functions of other insects and plants, it is essential to examine the role of ant species in the context of their trophic food web. Stable isotope analysis makes this possible, as this technique allows the tracing of nutrients, such as nitrogen, through a food web and the linking of trophic levels (Bluthgen *et al.*, 2003; Feldhaar *et al.*, 2010; Boecklen *et al.*, 2011; Menzel *et al.*, 2012; Kaspari *et al.*, 2017; Suehiro *et al.*, 2017). The isotope ^{15}N becomes enriched in the tissues of animals with increasing trophic level (on the order of ~3–5% per trophic transfer; DeNiro & Epstein, 1981). Ant species vary in trophic level, and stable isotope analysis can reveal much about a colony's position within the trophic web of an ecological community (Tillberg *et al.*, 2006).

Although studies have focused on the assessment of stable isotopes within arthropod trophic levels and effects of ants on soil conditions (Farji-Brener & Werenkraut, 2017; Iakovlev *et al.*, 2017), studies of correlations between ant nest presence and surrounding plant communities have not often employed stable isotope analyses (Beattie & Culver, 1977; Culver & Beattie, 1983; Sagers *et al.*, 2000; O'Grady *et al.*, 2010; Romero *et al.*, 2015; Iakovlev *et al.*, 2017; Roeder & Kaspari, 2017). Few studies combine soil, vegetation, and nitrogen stable isotope measurements in an assessment of the effects of individual ant colonies on their microhabitats.

In this study, we examine these processes in *Formica podzolica* (Francoeur, 1973), a mound-building, medium-sized, black ant that lives throughout high-altitude regions of North America. *Formica podzolica* are omnivorous, with a diet that includes nectar and honeydew as well as small arthropods and scavenged materials (Deslippe & Savolainen, 1995; Mooney & Tillberg, 2005). Petry *et al.* (2012) found that excess carbohydrates relative to protein in the diet may trigger *F. podzolica* aggressiveness, patterns of predation, and foraging activity. *Formica podzolica* nest locations correlate directly with natural food availability. Deslippe & Savolainen (1995) found nest densities and reproductive yield to be highest along forest edges (where natural food levels were high) and lowest in overgrazed meadows. *Formica podzolica* feed, on average, at or slightly above the trophic position of a primary predator (Mooney & Tillberg, 2005).

With their widespread distribution, large mound nests, and omnivorous foraging activity, *F. podzolica* probably plays an essential role in its broader ecosystem dynamics. In one of the first studies on ant interactions with soil and plant ecosystems, Beattie & Culver (1977) presented findings from soil and vegetation data (mainly including grass and juniper) from land immediately surrounding *Formica obscuripes* (Forel, 1886) mound nests at three different sites and measured plant species richness, diversity, and abundance. *Formica obscuripes* is similar in habitat and range to *F. podzolica*; both species are omnivores that harvest honeydew from aphids, build mound nests made of soil and plant material, and live in a wide variety of ecosystems across North America (Beattie & Culver, 1977). They found that, although plants were scattered throughout the study site, most plant species were positively associated with the location of mounds nests, and species abundance reached a peak at 1.5 m from nests in juniper-dominated areas. Additionally, nutrient-enriched soil correlated with plant species diversity. In a second study, Culver & Beattie (1983) found many of the same effects of an ant that is ecologically similar to *F. podzolica*, *Formica canadensis* (Santschi, 1914), on soils and vegetation at the Rocky Mountain Biological Laboratory in Gothic, Colorado. Beattie & Culver (1977) and Culver & Beattie (1983) inspired our examination of the ecological effects of the similar and abundant ant species *F. podzolica*. Our preliminary observations of *F. podzolica* colonies suggested higher vegetation density surrounding the nests than at greater distances from the nests, but that few plants grow on the nests themselves (Fig. 1a).

We hypothesised that *F. podzolica*, through their construction, foraging, and waste management, modify the ecosystem around each colony in ways that affect plant community structure. In areas surrounding *F. podzolica* nests, we analysed plant abundance, biomass, and ground cover, soil moisture and pH, and ^{15}N content of ants and plants. We predicted: (i) that plant abundance, diversity, biomass, and percentage vegetation cover would be greatest close to nests; (ii) that soil moisture would increase and pH would decrease with distance from nests; and (iii) that plant ^{15}N would be greatest close to nests. These predictions are based on evidence from other studies showing that ants create aerated, nutrient-enriched soils that favour plant growth and alter soil pH through excretions of uric acid (Folgarait, 1998; Wagner *et al.*, 2004; Sousa-Souto *et al.*, 2012). We provide an integrated set of insights into how this subalpine ant affects its immediate surroundings, and we find significant local patterns of plant community composition and soil moisture around nests. Few studies have provided such an integrated view of the interaction of ants with surrounding plant communities. These results support our understanding of the vital role of ants as ecosystem engineers, providing evidence of how they effectively modify microhabitats around them (Jones *et al.*, 1994).

Materials and methods

Study sites and location

Our study site, the University of Colorado's Mountain Research Station, is located at an elevation of 2900 m near

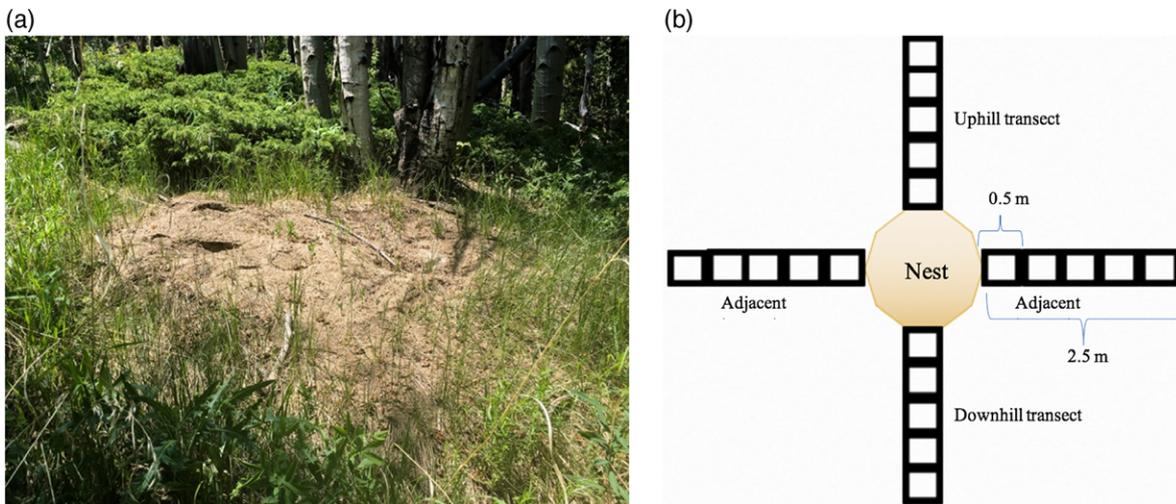


Fig. 1. (a) A representative *Formica podzolica* mound nest (photograph: MAS). (b) Transect layout around each nest. Each transect contained five 0.5×0.5 -m quadrats. [Colour figure can be viewed at wileyonlinelibrary.com].

Nederland, Colorado. We collected data in meadows and lodgepole pine-dominated forests around the Mountain Research Station. We used a total of 24 nests for data collection at similar elevation (2965–3000 m): nine in Elk Meadows (subalpine meadows dominated by grasses and forbs and largely void of trees; $40^{\circ}01' \text{ N } 105^{\circ}32' \text{ W}$), nine in areas off the Sourdough Trail (a hiking trail through a closed-canopy pine forest with limited undergrowth diversity; $40^{\circ}02' \text{ N } 105^{\circ}31' \text{ W}$), and five near C1 Meteorological Station (subalpine meadows dominated by grasses and forbs and largely void of trees; $40^{\circ}02' \text{ N } 105^{\circ}32' \text{ W}$). We took measurements and collected ant, plant, and soil samples from June to August 2016. Ant and plant samples were prepared at University of Colorado, Boulder, and sent to University of California Davis Stable Isotope Facility for stable isotope analyses.

Study organism

Formica podzolica nests occur in pine and aspen stands in Colorado and throughout the Rocky Mountain region, at altitudes up to 3000 m. This species constructs conspicuous soil mounds, which can exceed 2 m in diameter and estimates of colony size range from 5000 to 100 000 workers (Deslippe & Savolainen, 1995). Workers tend aphids for honeydew, scavenge, and prey on a wide variety of invertebrates from March to October (Deslippe & Savolainen, 1995). Winged, sexual individuals mate outside their nests from July to September, after which males die and females start new colonies (Deslippe & Savolainen, 1995). About one-third of *F. podzolica* colonies contain a single queen, while most have more than one queen, with low average nestmate relatedness (DeHeer & Herbers, 2004).

Plant abundance, biomass, and percentage vegetation cover

At each nest, we determined plant species abundance within 20 quadrats (each quadrat was 0.5×0.5 m) surrounding the

nest: five quadrats along a 2.5-m transect on the uphill, downhill, and two lateral slopes to the nest. Each transect started at the edge of the nest and ran linearly along or perpendicular to the aspect of the slope (Fig. 1b). For each nest, we recorded the number of stems per each plant species in each quadrat, as well as the degree of the slope.

We clipped all vegetation above ground from one randomly chosen quadrant from each quadrat (we cut a 0.25×0.25 -m area of vegetation per quadrat) at locations of 0.5 and 2.5 m from nests on the uphill and downhill slopes. We immediately dried vegetation in an oven at 60° C for 24 h to constant mass and measured the dry weight. We estimated percentage vegetation cover by laying a 10×10 grid over each quadrat and counting the number of squares containing vegetation.

Soil moisture and pH

We collected soil samples from each quadrat at all nests during June 2016 within a week of the last rainfall. We took samples from a depth of approximately 10 cm (Wagner *et al.*, 2004) using a trowel and sieved (2 mm). We immediately measured wet weight and then dried samples in an oven at 105° C for 24 h. To determine moisture content, we then measured dry weight and calculated the difference between the two weights. After making a suspension of soil in deionised water, we determined pH of each sample using a Mettler Toledo S20 SevenEasy pH meter (Columbus, Ohio).

Stable isotope analysis for ^{15}N content

To create a homogenised mixture of plant material, we ground plant samples taken from uphill and downhill transects at 0.5 and 2.5 m from nests using a ball mill. We collected 20 ants from each nest during the summer and froze them for preservation. We packed the ground plant samples and ants into tin capsules

(one plant tin per quadrat and two ant tins per nest) and sent them to UC Davis Stable Isotope Facility for ^{15}N analysis. By using an elemental analyser interfaced to a continuous-flow isotope ratio mass spectrometer, the facility determined the amount of ^{15}N for each sample. $\delta^{15}\text{N}$ is the difference between the sample atom percentage and a widely used natural abundance standard.

Statistical analysis

We performed all statistical analyses in R, version 3.4.1 (R Core Team, 2017). We used general or generalised linear mixed effects models (LMMs or GLMMs, respectively) to analyse all response variables, allowing random intercepts for each ant colony (nest) nested within site, and possible fixed effects of transect and distance from the ant nest (of each datum's quadrat). For pH and $\delta^{15}\text{N}$, general linear models on untransformed data were appropriate, while we analysed square-root-transformed data for percentage cover and above-ground plant biomass. We implemented these models using the *nlme* package (Pinheiro *et al.*, 2017). Transformations were insufficient or inappropriate for soil moisture and total plant abundance (number of stems), so we used the *lme4* package to implement GLMMs in these cases (Bates *et al.*, 2015), with a gamma distribution for soil moisture and a negative binomial distribution for plant count data, which was overdispersed. For each response variable, we evaluated a full model with distance, transect, and their interaction as possible fixed effects. If the interaction was not significant, we removed this term first. We then singly removed the other predictors in a stepwise manner until all predictors included had significant effects on the response variable. In additional analyses for $\delta^{15}\text{N}$, we evaluated the differences between ants and plant leaves (with a linear mixed model), and among sites (with ANOVA). We used residuals diagnostics on all models to assess model fit and appropriateness. For the generalised models (gamma for soil moisture and negative binomial for plant abundance), we assessed randomised quantile residuals (Dunn & Smyth, 1996) using the *DHARMA* package (Hartig, 2017). Data organisation, visualisation, and figure preparation were completed using the *tidyverse* set of packages (Wickham, 2017).

Details for all final statistical models, including information about model specification as well as random and fixed effects, are included in Table S1. We report estimates of coefficients on generalised or transformed scales, where applicable.

Results

Plant abundance, biomass, and percentage vegetation cover

When evaluating the number of plants per quadrat, there was a significant interaction between distance from the nest and transect (slope position). The number of plants decreased at greater distances from an ant nest for the downhill and adjacent transects, but this effect was not present in the uphill transects, as can be seen from the differing predicted slopes in Fig. 2a [negative binomial GLMM; distance effect for adjacent

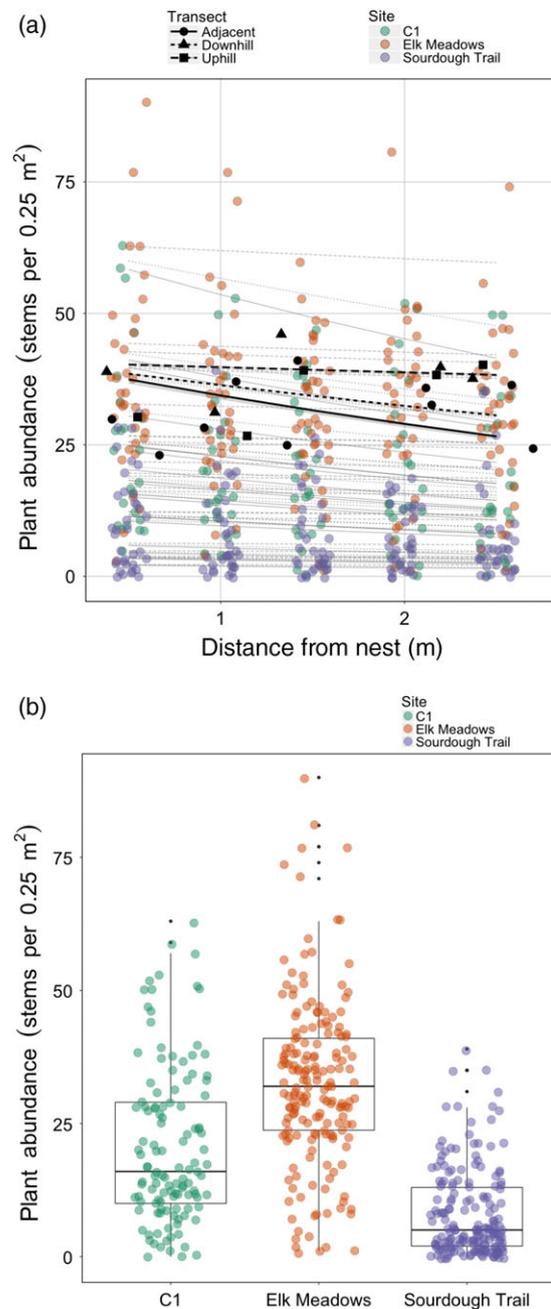


Fig. 2. (a) Plant abundance decreases with distance from ant nests in adjacent and downhill transects, but not in uphill transects. Points show jittered raw data, while lines show fitted model predictions. Grey open circles and grey lines show all data and model predictions, while black points and lines show an example for a single nest (EM3), to demonstrate the interaction. Solid lines and black circles show adjacent transects, small-dashed lines and black squares show downhill transects, and larger-dashed lines and black triangles show uphill transects. (b) Plant abundance is greatest at Elk Meadows and least at the Sourdough Trail site. Coloured dots show jittered values for each sample. Boxes include the middle 50% of the data, with the bar at the median value, and whiskers extend to the lowest and highest values that are within 150% of the interquartile range; small black dots are points outside that range. [Colour figure can be viewed at wileyonlinelibrary.com].

Table 1. Total number of stems for each plant species within 0.5-, 1-, 1.5-, 2-, and 2.5-m quadrats.

	0.5 m	1 m	1.5 m	2 m	2.5 m
<i>Muhlenbergia montana</i>	163	239	149	165	161
<i>Lupinus argenteus</i>	41	77	93	81	71
<i>Fragaria virginiana</i>	66	58	87	75	93
<i>Juncus arcticus</i>	1610	1095	1036	1040	902
<i>Sedum lanceolatum</i>	0	4	2	1	1
<i>Taraxacum officinale</i>	20	23	33	21	18
<i>Rumex acetosella</i>	60	90	79	81	113
<i>Thermopsis divaricarpa</i>	102	165	147	154	142
<i>Pseudocymopterus montanus</i>	12	19	24	18	15
<i>Juniperus communis</i>	4	5	4	4	4
<i>Abies lasiocarpa</i>	0	0	1	2	13
<i>Aquilegia coerulea</i>	0	0	1	0	0
<i>Achillea lanulosa</i>	86	97	97	118	141
<i>Populus tremuloides</i>	4	3	5	15	8
<i>Antennaria rosea</i>	0	2	9	9	5
<i>Campanula rotundifolia</i>	8	24	37	13	16
<i>Pentaphylloides floribunda</i>	74	74	58	69	49
<i>Stachys byzantina</i>	10	6	6	16	7
<i>Artemisia ludoviciana</i>	0	2	3	3	5
<i>Castilleja sulphurea</i>	17	9	6	7	14

transects: $\beta = -0.17$, $P < 0.0001$; distance interaction in downhill transects (difference in slope from adjacent): $\beta = 0.056$, $P = 0.15$; distance interaction in uphill transects (difference in slope from adjacent): $\beta = 0.15$, $P < 0.001$; see Table S1 for additional details]. Plant abundance was at its greatest at Elk Meadows and at its lowest at the Sourdough Trail site (Fig. 2b). Overall, the quadrats 0.5 m from nests contained a high abundance of plants that grow well in dry soil conditions, such as *Juncus arcticus* and *Pentaphylloides floribunda*. Quadrats 2.5 m from nests presented a higher proportion of large, fruiting plants such as *Fragaria virginiana* (a strawberry). Five species (*Fragaria virginiana*, *Juncus arcticus*, *Pentaphylloides floribunda*, *Abies lasiocarpa*, and *Achillea lanulosa*) showed a substantial response as measured by stems per quadrat in relation to distance from the ant mound (Table 1).

For percentage plant cover, there was no significant interaction or main effect of distance, but transect had a significant effect, as uphill transects had significantly lower percentage cover than did either downhill or adjacent transects [Fig. 3; LMM; downhill (difference from adjacent): $\beta = 0.078$, $P = 0.63$; uphill (difference from adjacent): $\beta = -1.17$, $P < 0.0001$; see Table S1]. Similarly, there was a significant effect of transect but not distance (or an interaction) on above-ground biomass, with uphill transects having significantly lower biomass than downhill; we did not measure biomass for adjacent transects [Fig. 4; LMM; uphill transect (difference from downhill): $\beta = -1.00$, $P < 0.0001$; Table S1]. Qualitatively, there are site differences in plants, as the samples from the Sourdough Trail site have consistently lower percentage plant abundance, cover, and biomass (Figs 3 and 4). Site acted as a random effect on intercept in our model; the intercept for plant abundance was 2.89 (intercept SD among sites = 0.66), that for plant cover was 4.96 (1.82), and that for biomass was 2.29 (0.57).

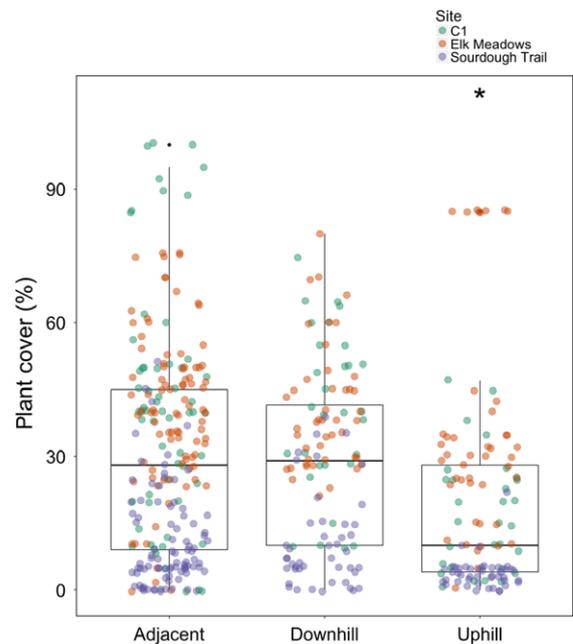


Fig. 3. Plant cover is lower uphill of ant nests than downhill or adjacent to ant nests. Coloured dots show jittered percentage cover values for each site. Boxes include the middle 50% of the data with the bar at the median value, and whiskers extend to the lowest and highest values that are within 150% of the interquartile range; small black dots are points outside that range. * indicates significant difference. [Colour figure can be viewed at wileyonlinelibrary.com].

Soil moisture and pH

Soil moisture increased with distance from ant nests [Fig. 5; gamma GLMM (log link); distance: $\beta = 0.18$, $P < 0.0001$, Table S1]. Transect was also a significant predictor of soil moisture [downhill (difference from adjacent): $\beta = -0.30$,

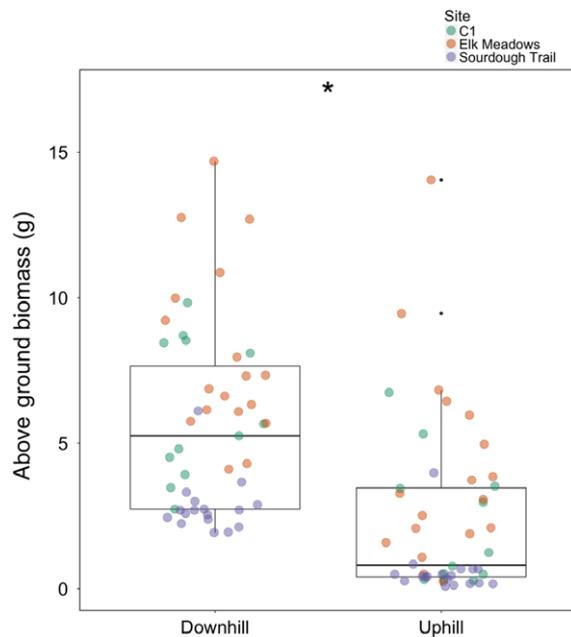


Fig. 4. Plant above-ground biomass is higher downhill of ant nests than uphill. Coloured dots show jittered biomass for each site. Boxes include the middle 50% of the data, with the bar at the median value, and whiskers extend to the lowest and highest values that are within 150% of the interquartile range; small black dots are points outside that range. * indicates significant difference. [Colour figure can be viewed at wileyonlinelibrary.com].

$P < 0.0001$; uphill (difference from adjacent): $\beta = -0.24$, $P < 0.0001$], but not the interaction. Transects adjacent to ant nests had higher soil moisture than did either uphill or downhill transects. Neither distance nor transect significantly predicted soil pH, and the final pH model was the null model (LMM; no fixed effects were significant predictors; Table S1).

¹⁵Nitrogen content

As expected, ant samples had significantly higher ¹⁵N content than did samples from plant leaves, as measured by $\delta^{15}\text{N}$ [LMM; plant leaves coefficient (difference from ants): $\beta = -4.56\%$, $P < 0.0001$; Table S1]. The mean $\delta^{15}\text{N}$ for ants across all sites was 2.98‰ (SE = 0.14‰, $N = 47$) and that for plants was -1.55‰ (SE = 0.19‰, $N = 125$). However, we did not find that plants closer to ant nests have higher $\delta^{15}\text{N}$, as neither distance nor transect significantly predicted $\delta^{15}\text{N}$ (LMM; no fixed effects were significant predictors; Table S1). ¹⁵N content in plant leaves did differ among sites, however, as plants at Sourdough Trail had significantly lower $\delta^{15}\text{N}$ than plants at either C1 or Elk Meadows (Fig. 6; ANOVA: $P = 0.002$; pairwise comparisons and additional details included in Table S1).

Discussion

The results of this study show that *F. podzolica* significantly change their microhabitat in a pattern that is consistent with

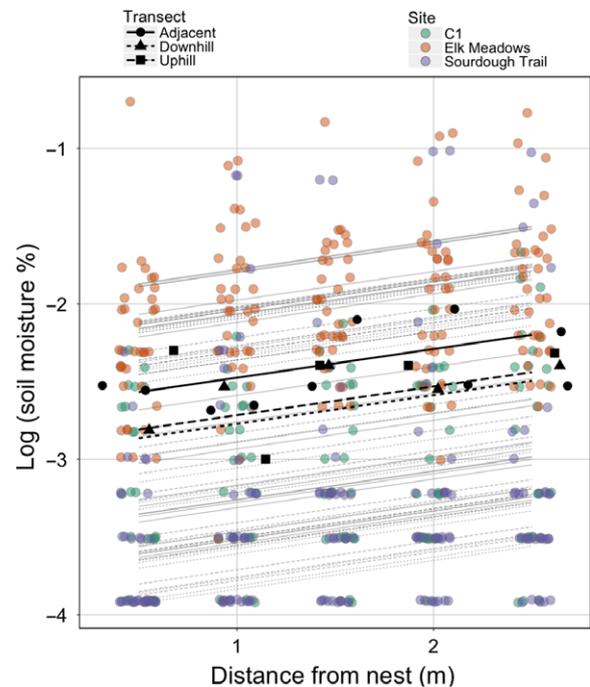


Fig. 5. Soil moisture (shown on log scale) increases with distance from ant nests and is higher adjacent to nests than uphill or downhill of nests. Points show jittered raw data while lines show fitted model predictions. Grey open circles and grey lines show all data and model predictions, while black points and lines show an example for a single nest (C1–2). Solid lines and black circles show adjacent transects, small-dashed lines and black triangles show downhill transects, and larger-dashed lines and black squares show uphill transects. [Colour figure can be viewed at wileyonlinelibrary.com].

other ant species (Jones *et al.*, 1994; Farji-Brener & Werenkraut, 2017). Plant abundance and soil moisture around the 24 nests have an inverse relationship (plant abundance decreases, and soil moisture increases, with distance from nest). Areas directly surrounding nests have greater plant abundance (except uphill), but they are also drier, perhaps due to uptake of water by plant roots. As a result, there is substantially different plant species composition near the nests than in non-nest soil. These results agree with previous studies that found soil directly under mound nests to be porous and relatively dry (Scherba, 1959; Laundré, 1990).

As we might predict from the soil moisture results, we found a pattern of dry-soil-dwelling plants near the nest (such as *J. arcticus* and *P. floribunda*; Stevens *et al.*, 2012) and plants requiring moister conditions more distant from the nest (e.g. *Achillea lanulosa*). *Lupinus argenteus* (lupine) had a pattern of decreasing abundance, same as *Collomia linearis* (trumpet) studied by Beattie & Culver (1983). These patterns suggest that the lower soil moisture directly surrounding nests contributes to a more optimal growing environment for grasses than for flowering plants like lupines and trumpets. We observed a slight qualitative increase in plant species diversity with distance from the colony, suggesting that dry soil conditions may reduce plant species richness in this habitat.

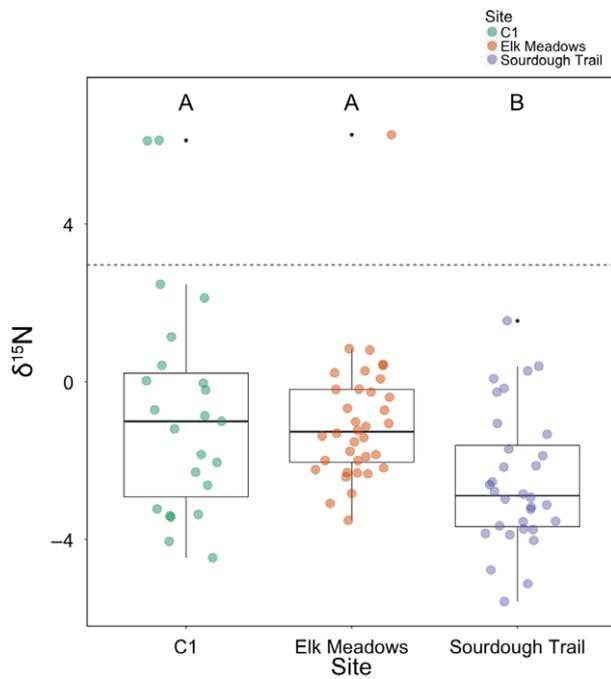


Fig. 6. Plants at the Sourdough Trails site have significantly lower ^{15}N than plants at either of the other sites. The horizontal dashed line shows the mean $\delta^{15}\text{N}$ of ant samples. Coloured dots show jittered values for each sample. Boxes include the middle 50% of the data, with the bar at the median value, and whiskers extend to the lowest and highest values that are within 150% of the interquartile range; small black dots are points outside that range. Letters indicate Tukey's *post hoc* significant differences. [Colour figure can be viewed at wileyonlinelibrary.com].

The increase in biomass in areas surrounding nests can probably be attributed to a variety of factors identified in previous studies, including the addition of organic matter to soil just outside nests as a result of discarded carcasses and foraging behaviour (Beattie, 1989; Sun & Zhou, 2013) and the creation of a warm, aerated environment below the soil surface (Lesica & Kannowski, 1998). While we did not find significant, direct evidence for this from our stable isotope study, plants often absorb nutrients from waste materials accumulated by ant colonies and have even been known to develop specialised tubers for occupation by ants in a robust mutualistic relationship (Beattie, 1989). We observed refuse piles outside the studied nests, suggesting that *F. podzolica* ants are adding to soil nutrients in this way. Berg-Binder & Suarez (2012) found that the density and biomass of plants, in addition to the amount of available N and K, were greater on the edges of *F. obscuripes* mound nests than in control areas. Similar to Beattie & Culver's, 1977 study, their findings reveal ecosystem engineering of a closely related ant to *F. podzolica*. Corpse removal, including carrying dead nestmates outside and discarding them on refuse piles, is a common undertaking strategy in various ant species (Sun & Zhou, 2013; Zelagin *et al.*, 2018). In these ways, ants actively cycle nutrients and move soil such that their nests often become advantageous areas for plants to grow. A meta-analysis by Farji-Brener & Werenkraut (2017) showed that the fertiliser

effect by ant nests is higher in dry habitats than in grasslands or savannahs, further suggesting that the subalpine ants of our study might be leading to soil enrichment. Additionally, they found that omnivorous ants have a greater effect on green vegetation growth than other types of foragers (leaf cutters and granivores), further elucidating our plant abundance and biomass findings.

The soil moisture, plant abundance, biomass, and percentage vegetation cover results suggest that slope of the study site might be an important factor in the distribution of vegetation around *F. podzolica* nests. Both biomass and percentage vegetation cover are significantly greater in the downhill transects than in the uphill transects of the nest sites. A plausible explanation is that water and nutrients naturally flow downhill and settle through the force of gravity. Ants might also tend to travel downhill when discarding organic material from their nests, although future research is needed to confirm this explanation. At the transect level, there was no effect of distance on uphill plant abundance, possibly as a result of moisture from nests accumulating downhill and therefore having little effect on uphill plants. Surprisingly, though, soil moisture was greatest in adjacent transects.

The lack of significance of change in soil pH around *F. podzolica* nests is also surprising, as other studies have found an effect of ants on soil pH (e.g. Frouz & Jilková, 2008). This pattern could be a result of differences in the baseline soil pH among studies.

Many of our plant samples had lower $\delta^{15}\text{N}$ values than leaf samples from a tropical rainforest of Australia (mean $\delta^{15}\text{N} = 2.2 \pm 1.3\text{‰}$; Bluthgen *et al.*, 2003) and a mixed birch forest of Russia ($\delta^{15}\text{N}$ ranging from -1.2‰ to 2.2‰ ; Iakovlev *et al.*, 2017), indicating that nitrogen might be a primary limiting nutrient of the subalpine forest of this study (as has been shown in other United States forest ecosystems; Aber *et al.*, 1989). This limiting nutrient, in turn, might explain the lack of statistical significance of distance from nest in ^{15}N content of plants. Similarly, the ant samples had lower $\delta^{15}\text{N}$ values than those of *Formica aquilonia* (Yarrow, 1955) from the mixed birch forest of Russia ($\delta^{15}\text{N}$ ranging from 5.3‰ to 5.8‰ ; Iakovlev *et al.*, 2017), but higher $\delta^{15}\text{N}$ values than those of *Formica lemmani* (Bondroit, 1917) from a limestone grassland in Ireland (mean $\delta^{15}\text{N} = 2.12 \pm 0.19\text{‰}$; O'Grady *et al.*, 2010). The high $\delta^{15}\text{N}$ values of ants compared with the vegetation surrounding their nests indicates that they are consumers at a higher trophic level within the ecosystem (Bluthgen *et al.*, 2003). The difference between mean $\delta^{15}\text{N}$ for plants and ants found in this study (4.53‰) suggests an average one trophic level step between the plants and ants, emphasising the importance of nectar, honeydew, and seeds in this ant species' diet.

Nests near the Sourdough Trail site yielded substantially different measurements of $\delta^{15}\text{N}$, biomass, percentage vegetation cover, and plant abundance than did other sites. This is probably because this study site is situated in a rockier part of the forest than the other two sites and on an exposed ridge that is subject to higher rates of wind and erosion. We controlled for the effects of site in our LMMs in order to ascertain major effects. This intersite variation is interesting and suggests a strong interaction between substrate quality and the food web of these ants.

It is difficult to infer a relationship between nitrogen in ants and that of plants, due in part to the lack of soil stable isotope data in our study. Our test for evidence of an ant-derived nitrogen signal in plants near nests yielded negative results. Such a signal might exist in the soils, which we did not test. The stable isotope results suggest that *F. podzolica* is just one trophic step removed from the plants, making an effect on plant nitrogen more difficult to detect. Carnivorous animals produce more elevated nitrogen signals (Tillberg *et al.*, 2006) and might leave a stronger fingerprint in surrounding organisms. However, we did not find direct evidence that plant ^{15}N is affected by ant nests.

The subject of ‘cause and effect’ must be examined in this study; do *F. podzolica* ants directly affect on their surrounding vegetation, or do the ants deliberately choose nest sites that provide the conditions described in the results? It is impossible to say with certainty from our results, but our study and previous studies suggest the former. The significant relationships found between soil moisture, biomass, and plant cover data within the 2.5 m surrounding nests suggests that nests are an influential factor; it is very unlikely that the landscapes of C1 Meteorological Station, Elk Meadows, and the Sourdough Trail would contain pockets of nest-like soil and plant conditions, each having been discovered by an ant colony. There was no indication that nest areas should contain different soil types initially, such as shade or topography. This conclusion is supported by other research showing that ant colonies change and manipulate their broader environments, such as Frouz & Jilková’s, 2008 finding that daily activities of ants result in a shift of pH in the soil surrounding the nest towards neutral, and the work of Finér *et al.* (2012) that revealed an increase in nitrogen cycling in boreal forests of eastern Finland as a result of wood ant (*F. rufa*) activity.

Small organisms can have significant influences on their ecosystems, as is shown in our results. Although each nest is small compared with the area of the entire forest and we examined small-scale impacts around the nest ecosystem instead of whole landscape effects, *F. podzolica*’s collective impacts of altered soil moisture on vegetation structure are exemplary of their status as ecosystem engineers. A 2015 meta-analysis of 122 studies of ecosystem engineers showed that the general influence of ecosystem engineers on diversity is positive (Romero *et al.*, 2015). Our study supports this and builds on other ant–ecosystem interaction studies (Beattie & Culver, 1977, 1983), adding a combination of vegetation and soil measurements to the evidence that ants can cause change in their environments. We found that *F. podzolica* nests significantly alter aspects of their ecosystem, especially plant communities, affecting soil moisture and plant abundance, cover, and biomass.

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Author contributions

MAS contributed to project design, data collection and analysis, and paper writing. MDB contributed to project design and paper writing. HFM contributed to data analysis and paper writing.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Model details for final statistical models. For each response, the final model is reduced from a full model with distance from ant nests, transect, and their interaction as fixed effects, except where otherwise noted. Random effects, where applicable, include ant nest nested within site, acting on intercepts.

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