Ant-mediated ecosystem functions on a warmer planet: effects on soil movement, decomposition and nutrient cycling

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Summary

1. Direct and indirect consequences of global warming on ecosystem functions and processes mediated by invertebrates remain understudied but are likely to have major impacts on ecosystems in the future. Among animals, invertebrates are taxonomically diverse, responsive to temperature changes, and play major ecological roles which also respond to temperature changes.

2. We used a mesocosm experiment to evaluate impacts of two warming treatments ($+3.5$ and $+5$ °C, set-points) and the presence and absence of the ant *Formica subsericea* (a major mediator of processes in north temperate ecosystems) on decomposition rate, soil movement, soil respiration and nitrogen availability.

3. Replicate 19-L mesocosms were placed outdoors in lathe houses and continuously warmed for 30 days in 2011 and 85 days in 2012. Warming treatments mimicked expected temperature increases for future climates in eastern North America.

4. In both years, the amount of soil displaced and soil respiration increased in the warming and ant presence treatments (soil movement: 73–119%; soil respiration: 37–48% relative to the control treatments without ants).

5. Decomposition rate and nitrogen availability tended to decrease in the warmest treatments (decomposition rate: −26 to −30%; nitrate availability: −11 to −42%).

6. Path analyses indicated that ants had significant short-term direct and indirect effects on the studied ecosystem processes. These results suggest that ants may be moving more soil and building deeper nests to escape increasing temperatures, but warming may also influence their direct and indirect effects on soil ecosystem processes.

Key-words: climate change, decomposition, ecosystem processes, *Formica*, nitrogen availability, soil movement, soil respiration

Introduction

Most of our understanding of the importance of biological diversity for mediating ecosystem processes and services comes from research done using plants or microbial assemblages (Mace, Norris & Fitter 2012); the role of consumers in mediating ecosystem processes remains understudied (Hooper *et al.* 2005). Some invertebrates are known to have key direct and indirect roles in major

ecosystem processes (Bardgett & Wardle 2010). Field observations and experiments have illustrated that terrestrial invertebrates directly influence decomposition (e.g. shredding of detritus: Wall *et al.* 2008) and soil modification (e.g. burrow construction: Kuiper *et al.* 2013), thus indirectly affecting nutrient cycling and availability (Nielsen *et al.* 2011; Prather *et al.* 2012). These roles may change in magnitude and directionality because of climatic change, potentially leading to changes in ecosystem functions and services (*sensu* Cardinale *et al.* 2012). However, little is known about the effects of climatic change

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on invertebrate-mediated ecosystem processes and services (Staudinger *et al.* 2012; Grimm *et al.* 2013). In a recent review, Prather *et al.* (2012) suggest that climate change is likely to affect key ecosystem processes and services mediated by invertebrates (e.g. nutrient cycling, decomposition and habitat formation). Field observations have suggested that ants play major roles as soil bioturbators and can create or modify terrestrial ecosystem structure and function at various scales, but these observations have proven to be difficult to test experimentally (Prather *et al.* 2012). Our study enhances our understanding of ecosystem processes mediated by ants in a novel and simplified experimental design and specifically addresses the consequences of warming on key ant-mediated ecosystem processes.

Among ground-dwelling invertebrates, ants are an ideal study system to use in experiments designed to evaluate impacts of climatic warming on animal-mediated ecosystem processes at the air–soil interface. Ants can directly alter soil movement and decomposition rates and indirectly affect soil nutrient dynamics (Wardle *et al.* 2011; Del Toro, Ribbons & Pelini 2012; Kendrick *et al.* 2015), by their building of nests in the soil, shredding organic material and foraging in leaf litter. Ant activity, abundance and diversity also respond rapidly to local changes in temperature (Pelini *et al.* 2011). However, the additive or interactive effects of warming and ants on ecosystem processes remain largely unexplored.

Here, we describe results from a mesocosm experiment designed to assess how warming induces changes in the activity of the ant species *Formica subsericea* Say, 1836, that subsequently alter rates of soil movement, decomposition of leaf litter, soil respiration and nitrogen availability. We hypothesized that in warmer treatments, increased ant activity would lead to increases in (i) soil movement; (ii) active shredding of leaf litter and its decomposition; (iii) soil respiration rate; and (iv) nitrogen availability.

Materials and methods

EXPERIMENTAL DESIGN

Mesocosm experiments have been used extensively to test hypotheses derived from field observations that cannot be tested with large-scale field experiments because of logistic constraints (Sala *et al.* 2000) and can help simplify complex systems to identify the impact of individual manipulated variables on a constrained number of measured responses. Our mesocosm experiment was a two-way factorial experimental design with two levels of warming (+3·5 and +5 °C set-points) crossed with three treatments of soil manipulation: (i) addition of ants [*Formica subsericea*: $N = 96$ (2011); $N = 82$ (2012)]; (ii) undisturbed controls [$N = 15$ (2011); $N = 24$ (2012)]; and (iii) a disturbance control, in which every week the upper 3–5 cm of the top soil layer was manually overturned the with a trowel and the bottom soil layers were aerated with a 25-cm pick [$N = 12$ (2012 only)]. The disturbance control was established to evaluate the effects of manual soil manipulation in comparison with soil manipulation done by ants on the measured response variables. The experimental warming

temperatures that we used represented expected summer temperature increases for eastern North America by 2100 under two different climatic change scenarios (i.e. +3·5 °C, RCP6.0 scenario and +5·0 °C, RCP8.5 scenario; Deser *et al.* 2012; IPCC 2013). The experiment ran for 30 days in 2011 (June–July) and 85 days in 2012 (May–July). Projected increases in temperatures are likely to reach the critical thermal maxima (Oberg, Del Toro & Pelini 2012) of some common ant species of eastern North America (including *F. subsericea*) that can ultimately have negative impacts on the ant physiology and functionality of ants in temperate ecosystems.

Each mesocosm was contained in a 19-L plastic bucket (30 cm diameter and 39 cm height; Fig. 1a). The containers were filled with three layers of soil, which mimicked the soils where *F. subsericea* commonly occurs. The lowest layer consisted of 2·0 L of 1·2-cm sized gravel and 2·0 L of sand; the middle layer consisted of 5·5 L of sand and 2·0 L of gravel; and the top layer consisted of 2·0 L of sand and 2·0 L of heat-treated compost. We drilled eight 1-mm diameter holes at the base of each mesocosm to allow excess water to drain out. The top 2 cm of each container was coated with petroleum jelly to prevent the ants from escaping each mesocosm. We cut a 14-cm diameter hole on each of the mesocosm lids and covered the opening with 1-mm fibreglass mesh. The mesh allowed ambient humidity and the heat from the lamps to penetrate the mesocosm, while excluding potential predators and seeds and preventing the ants from leaving the mesocosm. Temperature sensors were placed in the middle of each soil layer, by drilling 5-mm diameter holes and inserting thermocouples into the mesocosm. The holes were then completely sealed with silicone (Fig. 1a). Mean, minimum and maximum hourly temperatures were recorded on a CR1000 datalogger (Campbell Scientific, Logan, UT, USA) and downloaded weekly. Humidity was recorded using a CD620 HydroSense water-content sensor (Campbell Scientific) and monitored three times per week to make sure that all treatments remained at a constant soil moisture (5–8% soil moisture content). If soil moisture in the mesocosms fell below 5%, they were watered for 5 min with a sprinkler irrigation system. Such an extreme reduction in soil moisture occurred only three times in the +5 °C warming treatment and once in the +3·5 °C warming treatment throughout the duration of the experiment.

The mesocosms were actively warmed using 250-watt infrared heat lamps (Phillips 250W, 120 volt heat light, Rosemont, IL, USA) (Fig. 1a). We chose infrared lighting because we believe this colour spectrum would not affect diurnal and nocturnal activity and behaviours in the ants. Previous work with the ant *Formica cuniculaia* (a closely related species of *F. subsericea* in the *fusca* group) shows this species to have a dichromatic visual system and is unable to distinguish colour differences at wavelengths >540 nm (Aksoy & Camlitepe 2012); therefore, we believe the infrared lighting was not detectable by the ants but the temperature manipulation was. To achieve a mean temperature increase of 3·5 °C, the lamps were hung 120 cm above the surface of the mesocosms. To achieve a mean temperature increase of 5 °C, the lamps were hung 60 cm above the surface of the mesocosms. Lamp bulbs were left on continuously so that daily temperature fluctuations were consistent across the treatments; bulbs were replaced as needed. One lamp evenly heated four mesocosms through all soil layers, so we clustered the mesocosms in groups of four; the distribution of the soil manipulation treatments was randomized within each temperature treatment. Actual hourly mean temperatures experienced within each mesocosm in
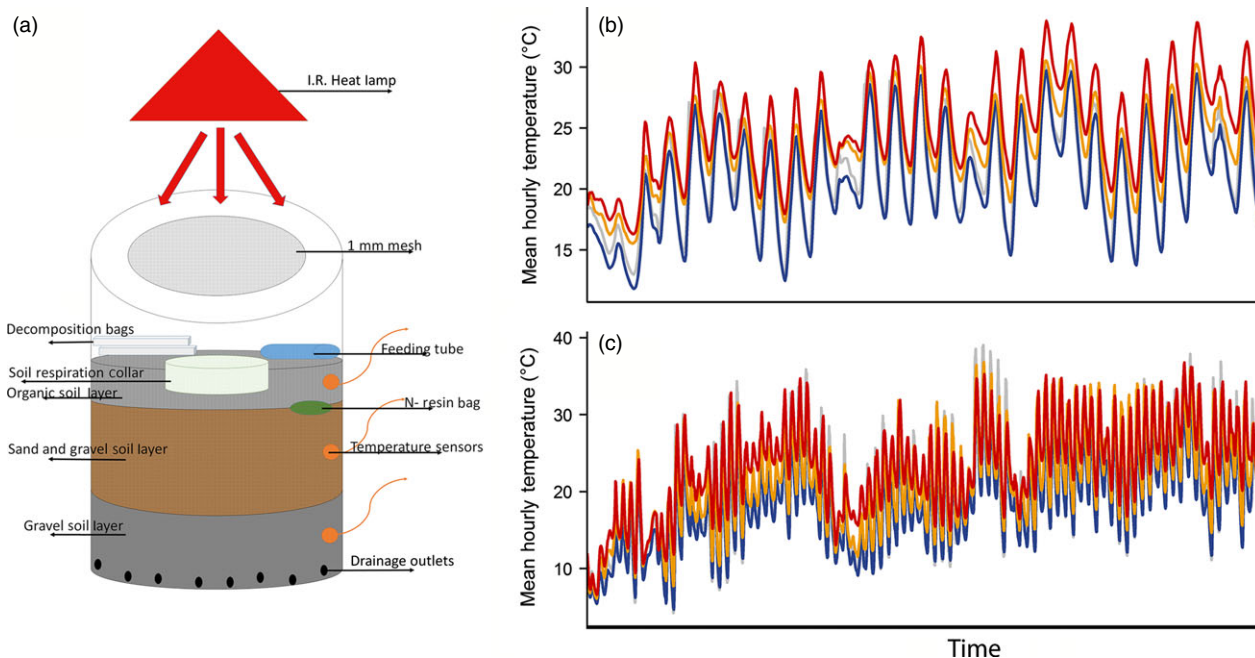
Fig. 1. Diagram of the mesocosm design and temperature variation in the three experimental warming treatments. (a) Mesocosm diagram (b) mesocosm temperature variation in 2011 season. Blue lines show the mean temperatures for the control treatment, orange lines show the mean temperatures for the +3_5 warming treatment, and red lines show the mean temperatures for the +5 treatment. Grey lines show the temperature data from the Harvard Forest Fisher Meteorological Station for the same dates. (c) Mesocosm temperature variation in 2011 season; coloured lines are the same as in (b).

the '+3·5 °C treatment' ranged from 0·5 to 3·9 °C warmer than controls, whereas the '+5 °C treatment' ranged from 1·7 to 6·7 °C warmer than controls (Fig. 1b,c), with the largest temperature differences occurring during the coolest and warmest times of the day. These soil temperatures are consistent with air and soil temperature variation observed at another Harvard Forest field site where open-top chambers are being warmed (Pelini *et al.* 2011) to replicate IPCC warming projections and at the Fisher meteorological station in Harvard Forest (Fig. 1b,c, grey line) which documents daily temperature variation.

THE ANT

Formica subsericea is a common and widely distributed species that builds large nests in soil. Its large colonies (hundreds to >10 000 workers) typically are found in edge and open habitats throughout eastern North America (Ellison *et al.* 2012). The genus *Formica* has a Holarctic distribution, and many of the species in the diverse and widespread *fusca* group (which includes *F. subsericea*) share similar natural history traits (Francoeur 1973; Gösswald 1989). We expect, therefore, that the results from our experiment are likely to apply to other north temperate zone soils where *Formica fusca*-group species occur. We collected ant colonies from sandy soils at three localities in central Massachusetts: the Montague Pine Barrens (42·569° N, −72·536 °W), the Devens Pine Barrens (42·452° N, −71·641 °W) and Myles Standish State Forest (41·839° N, −70·691 °W). Each mesocosm was established with 100 workers from independent ant colonies; no queens were collected or used in the mesocosms. We expected that individual activity and behaviour would not be affected by the absence of queens as ants self-organize tasks without the need of direction from a reproductive caste (Oster & Wilson 1978; Gordon 2010).

To keep the ants alive for the duration of the experiment, the ants in each mesocosm were fed with a 5% sugar and 5% glutamine solution, which mimics honeydew, stored in a 50-ml vial and replaced on a weekly basis (Fig. 1a). The aqueous solution was contained in the vials with a cotton ball and did not in any way leach into the soil filling the mesocosm. We counted and removed the number of dead workers in each mesocosm on a weekly basis. Mortality rates ranged from 10 to 22 ants per mesocosm, and there were no significant differences in mortality rates across treatments or between years.

ECOSYSTEM PROCESSES

We measured mass loss (as an indication of decomposition rate) by placing two fibreglass mesh bags filled with leaf litter on top of the soil in each mesocosm. Each bag contained 1 g of dried (70 °C for 72 h) whole red maple (*Acer rubrum* L.) leaves + 1 g of dried whole red oak (*Quercus rubra* L.) leaves. One litter bag excluded ants because of the size of the mesh (1 mm), while the second bag allowed ants access to the litter (mesh size = 10 mm). At the end of the experiment, litter bags were removed from the mesocosms; the remaining litter was dried (70 °C for 72 h) and weighed (±0·001 g), to determine mass lost. At the end of the experiment, the 10-mm-mesh bags contained much smaller portions of leaves and often had ants still inside the bags when opened (personal observation), whereas no ants were ever detected in the 1-mm-mesh bags.

Soil respiration (as CO_2 flux) was measured for 5 min every 7 days in 2011 and every 14 days in 2012 using a portable infra-red gas analyzer (LI-COR 6400; LI-COR Biosciences, Lincoln, Nebraska) with a soil-flux chamber (LI-COR 6400-09) placed on a PVC collar (20-cm diameter, 5-cm deep) half-buried at the soil

surface in a subset of the mesocosms [N = 38 (2011); N = 72 (2012)]. We used linear interpolation and integration (based on the relationship between measured soil temperature and CO_2 flux; Savage, Davidson & Tang 2013) to estimate the amount of CO_2 produced per day over the course of the experiment each year.

We determined the total amount of available nitrogen (NH_4^+ and NO_3^-) as that captured on ion-exchange resin bags placed in each mesocosm (Maynard, Kalra & Crumbaugh 2008). Approximately 5 g of resin was placed in a nylon mesh bag and pretreated with 2 mol L^{-1} KCl before being placed in the mesocosms. Resin bags were placed 3 cm below the surface of the soil and left in the mesocosm for the duration of the experiment during the 2012 season (Fig. 1a). Resins were returned to the laboratory and dried at 105 °C for 24 h. Resins were then extracted in 100 mL of 1 mol L^{-1} KCl for 48 h. Resin extracts were filtered through a coarse pore filter (0·45–0·60 μm), and inorganic N concentrations were determined colorimetrically with a Lachat AE flow-injection autoanalyzer (Lachat Instruments, Inc., Loveland, CO, USA) using the indophenol-blue and cadmium reduction methods for NH_4^+ and NO_3^-, respectively. Nitrogen mineralization is a reliable predictor of soil productivity (Raison, Connell & Khanna 1987), and total nitrogen captured on resins is closely correlated with nitrogen mineralization (Strader, Binkley & Wells 1989). Net nitrogen capture was determined by subtracting the concentration of inorganic nitrogen (NH_4^+ + NO_3^-) in the incubated samples from that of the initial samples (Morecroft, Marrs & Woodward 1992). High net nitrogen capture can be indicative of high levels of microbial activity in the soil (Chapin, Matson & Mooney 2002).

We estimated the amount of soil moved by *F. subsericea* by sacrificing the colony at the end of the experiment and creating a three-dimensional paraffin cast of the nest (Gulf Wax®; Royal Oak, Roswell, GA, USA) as described by Tschinkel (2010). We estimated the volume of soil displaced from the weight of the cast and the density of the paraffin (0·9 g cm^{-3}). We also recorded the maximum depth (cm from the surface) for every cast.

DATA ANALYSIS

We used analysis of variance (ANOVA) and post hoc Tukey pairwise comparisons to test the effects of warming and different forms of soil alteration (predictor variables) on the direct and indirect ecosystem processes of soil movement, decomposition rate, soil respiration and nitrogen availability (response variables) (R code, data sets and detailed ANOVA outputs are in Appendix S1, Supporting information). We divided the processes into two categories, those directly mediated by ants and indirect effects expressed as the ant × treatment interaction. We defined direct processes as those where the ants came in direct contact with the substrate (e.g. soil moved or litter shredded) and indirect processes as those where the consequence of a direct effect altered the process in question (e.g. soil respiration is influenced by soil moved, and nitrogen capture is influenced by shredded or decomposed litter).

We used partial least squares path modelling (plspm) analysis to evaluate the direct and indirect effects of warming and the presence and absence of ants on soil movement, decomposition, soil respiration and nitrogen availability [plspm package (Sanchez 2013) of R version 3.03 (R Core Development Team 2014) code and detailed outputs in Appendix S1]. Experimental replication and statistical power was higher for the ant addition and unmanipulated control treatments, and these were used in the plspm analysis.

Results

DIRECT EFFECTS OF ANTS ON ECOSYSTEM PROCESSES

In 2011, soil surface temperatures ranged from 11 to 31 °C in the control treatment, whereas the soil surface in the warmest treatment reached a maximum of 38 °C. In 2012, soil surface temperatures ranged from 5 to 32 °C in the controls and reached a maximum of 40 °C in the high-temperature treatment (Fig. 1b,c). In both 2011 and 2012, ants displaced significantly more soil (P < 0·001; Figs 2a,b) and built deeper nests in the warmer treatments than in the controls [$\bar{x}$ = 15·5 ± 0·8 (SD) cm in the controls; 22 ± 1·2 cm in the +3·5 °C treatment; 23 ± 1·2 cm in the +5 °C treatment, P < 0·01 (2011); and P = 0·03 (2012)]. Ants actively foraged inside the 10-mm decomposition bags, shredding leaf litter and carrying leaf particles back to the nest, but we did not observe similar foraging in the 1-mm-mesh bags. Decomposition was higher in the 10-mm-mesh bags than the 1-mm-mesh bags (44% more litter loss in 2011 and 61% more litter loss in 2012; P < 0·001), and litter loss decreased with increasing temperature [Fig. 2c,d; 84% decrease, P < 0·0001 (2011); 52% decrease, P = 0·002 (2012)]. In 2011, there was a significant ant × warming interaction in leaf-litter-mass lost, with less litter lost in the warming treatments (Fig. 2c). In 2012, this interaction was not significant, but the trend was in the same direction (Fig. 2d, P = 0·09). Decomposition was highest in the controls in 2012, and there were no significant differences among the ant addition or undisturbed warming treatments (Fig. 2d).

INDIRECT EFFECTS OF ANTS ON ECOSYSTEM PROCESSES

In both 2011 and 2012, the presence of ants was associated with an ≈25% increase in soil respiration rate [P < 0·01 (2011); P < 0·05 (2012); Fig. 2a,b]. In contrast, we observed an effect of warming on soil respiration rate only in 2012: soil respiration rate was 38% higher in the +3·5 °C treatment and 28% higher in the +5 °C warming treatment than in the control [P = 0·30 (2011); P < 0·01 (2012); Fig. 2a,b]. In 2011, there also was a significant ant × warming interaction (Fig. 3a), but this interaction was not observed in 2012 (Fig. 3b).

Ammonium (NH_4^+) concentration in mesocosms with ants decreased 74% as temperature increased (P < 0·01, Fig. 3c), but there were no effects of temperature on NH_4^+ concentrations in the empty mesocosm controls. There was a significant soil treatment × warming interaction: as NH_4^+ concentrations in the ant mesocosms decreased in the warmer treatments, NH_4^+ concentrations in controls increased (P < 0·01, Fig. 3c).
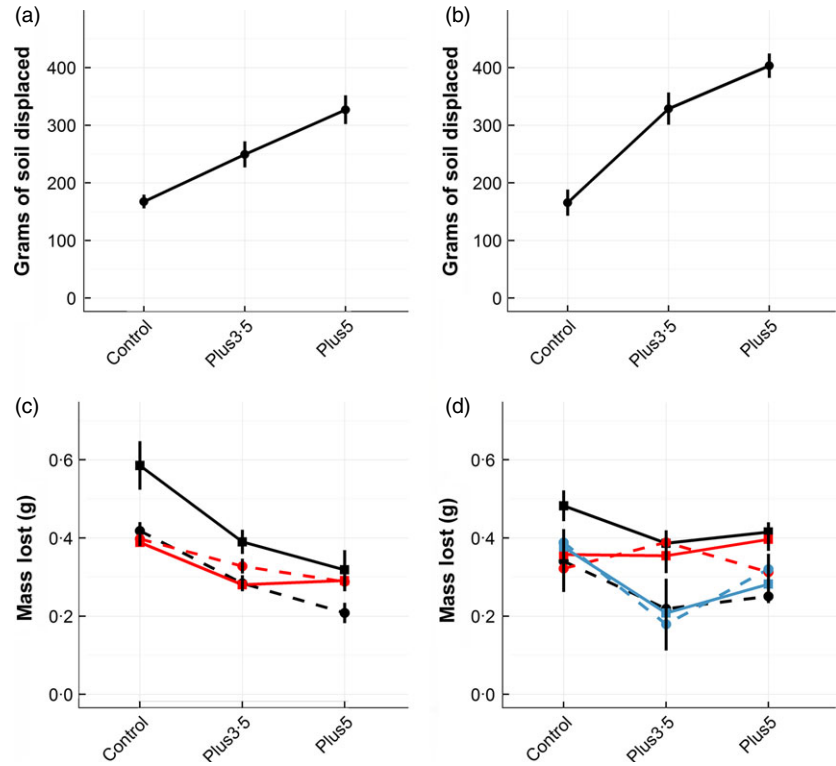
Fig. 2. Interaction plots (as in Gotelli & Ellison 2013, chapter 10) of the direct effects of ants on soil movement (A, B) and decomposition (C, D) in 2011 (A,C) and 2012 (B,D). Values shown are means ± 1 standard error of the mean. Line and symbol colors indicate ant additions (black); disturbance controls (blue); and undisturbed controls (red). In C and D, solid lines denote the 10-mm-mesh decomposition bags (ants had access to the litter) and dashed lines denote the 1-mm-mesh decomposition bags (ants did not have access to the litter).
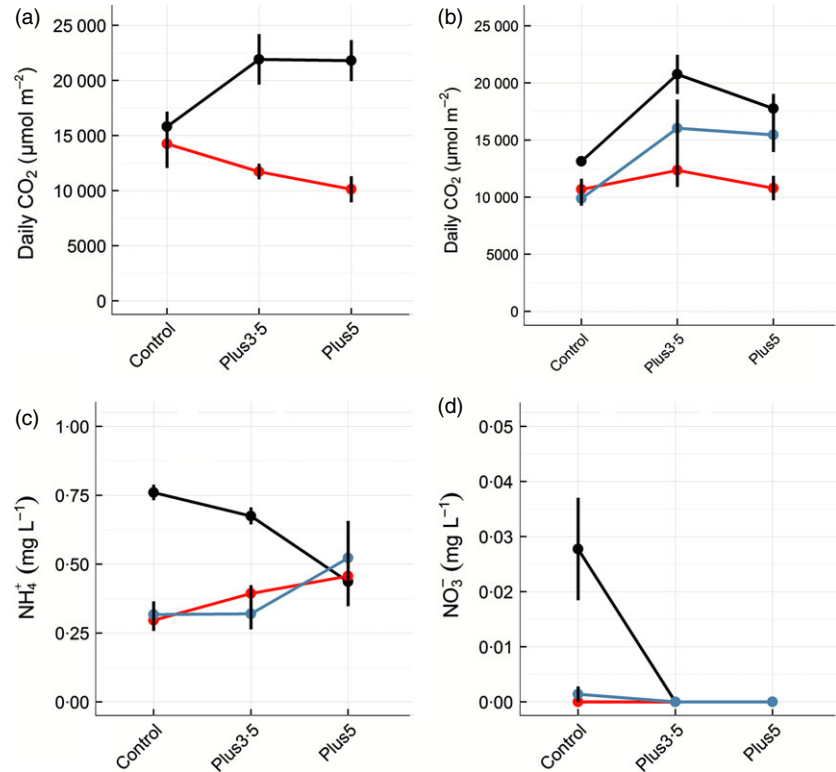

Fig. 3. Interaction plots of the indirect effects of experimental treatments on soil respiration (A: 2011; B: 2012) and nitrogen availability (C: NH_4 in 2012; D: NO_3 in 2012). Line and symbol colors indicate ant additions (black); disturbance controls (blue); and undisturbed controls (red).

Similarly, when ants were present, nitrates (NO_3^-) tended to decrease to undetectable amounts with warming ($P = 0.09$, Fig. 3d). Although NO_3^- concentrations were highest when ants were present in unheated mesocosms ($P = 0.04$; Fig. 3d), there were no differences in NO_3^- concentrations among mesocosms heated to either +3.5 or +5.0 °C.

Effects of ants, warming and their interaction on NH_4^+ and NO_3^- resulted in significant effects on total available nitrogen ($P < 0.01$, Appendix S1). In particular, when

ants were added to unheated mesocosms, nitrogen capture (a combination of mineralization and total nitrogen) was four times higher than in empty mesocosms and the disturbance controls. These differences were much less pronounced in warmed mesocosms and were driven largely by NH_4^+ concentrations.

PATH ANALYSES

In both years, warming had direct negative effects on litter decomposition but the presence of ants increased decomposition rates only in the 10-mm bags (Fig. 4a,b). Little of the variation in litter decomposition was explained by the ant × warming interaction in 2012 (Fig. 4b). Ants had strong direct effects on the amount of soil moved, which led to strong (indirect) effects of ants on daily CO_2 flux. The indirect effects of ants were greater than the effects of warming on CO_2 flux in 2012. Ants also had indirect effects on nitrogen availability: NH_4^+ concentration and NO_3^- were affected by the negative relationship with warming, and NO_3^- was positively affected by NH_4^+ concentrations (Fig. 4b).

Discussion

Global climatic changes are likely to impact ecosystem processes and services mediated by arthropods, but the cascading and interacting effects of climate change on arthropods and ecosystem functions remain understudied. Our experimental results in mesocosms support conclusions from field observations that ants can influence soil movement and soil respiration, at both current and projected temperatures (Domisch *et al.* 2006). However, the effects of ants on decomposition and nitrogen availability that are seen at current temperatures (e.g. Del Toro, Ribbons & Pelini 2012; Prather *et al.* 2012; Kendrick *et al.* 2015) are much reduced in warmer temperatures. As ants reach temperatures close to their critical thermal maximum, ant functional roles may change in response to thermal stress. We speculate that the reduc-

tions in decomposition and nitrogen availability could be due to reduced ant surface activity, but this merits further investigation. Because we used mesocosm experiments to simplify the interactions between ants and ecosystem function, our findings may not be entirely representative of natural systems, but rather show the potential influence of warming on processes mediated by ants.

Nest building serves various functions for ant colonies, one of which is hypothesized to be the thermoregulation for the worker ants and their brood (Jones & Oldroyd 2006; Kadochová & Frouz 2013). This hypothesis posits that nests are excavated to a size and depth where preferable temperatures for brood and workers exist. In our experimental mesocosms, ants built larger and deeper nests in heated soils, a finding that supports the thermoregulation hypothesis. *Formica subsericea* has a maximum thermal tolerance of 40 °C (Oberg, Del Toro & Pelini 2012), and an escape from heat is an important function of nests, especially during the hottest time of the year, when surface temperatures can exceed 40 °C (and when this experiment was conducted). This can potentially become an important issue as temperature is predicted to increase globally and heat waves occur at a higher frequency in future climates (IPCC 2013), thus leading to some ants building deeper nests to optimize nest thermoregulation.

Ants decomposed more leaf litter under ambient temperatures, and up to 84% less in warmer treatments. In fact, litter decomposition rates in the +5 °C mesocosms with ants were not different from litter decomposition rates in mesocosms without ants. Decreased decomposition in the warmed treatments may be attributable in part to desiccation caused by the infrared heat lamps, but the significant warming × soil treatment term also may indicate that ants reduced their surface activity in the warming treatments (see also Diamond *et al.* 2013), resulting in less litter shredding. Stuble *et al.* (2014) demonstrated that within experimental warming chambers at Harvard Forest and Duke Forest (which have similar temperature increases as our experiment) one metric of ant activity,
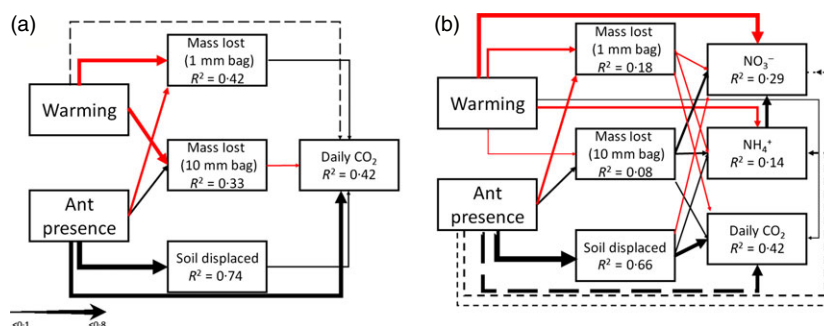

Fig. 4. Path analysis models for the 2011 (A) and 2012 (B) experiment years. Each box represents a measured predictor or response variable. Arrows indicate the hypothesized cause and effect relationship between two variables, and line width is scaled by the standardized loading coefficients for each connection for those with coefficients >0.10. Red lines indicate negative relationships, and black lines indicate positive relationships. Dashed lines indicate relationships for which indirect effects were stronger than direct effects. Coefficients of determination (R^2) are reported in boxes with response variables.

foraging, was not influenced by temperature increases. We suggest that ants directly affect decomposition by less actively shredding leaf litter and that ant-mediated decomposition rates may decline further under future warming conditions. This pattern may apply to other species in the genus *Formica* as they often use leaf litter material in their nest construction (Gösswald 1989; Kadochová & Frouz 2013).

In contrast, Wardle *et al.* (2011) observed that decomposition rate increased when ants were excluded. Our results could have differed from those presented in Wardle *et al.* (2011) because of unmeasured differences in soil quality and microbes. The sandy soils we used typically have low water retention and osmotic potential (Chowdhury, Marschner & Burns 2011) and species-poor microbial communities. The soil strata built in these mesocosms were meant to reflect similar soil conditions to those found in naturally occurring *F. subsericea* nests; however, it is likely that our soils are not a perfect replicate of field nest conditions due to the sterilization and soil disturbances necessary to build the mesocosms. Wardle *et al.* (2011) completed their work in the field, with moister forest soils that support a much richer soil microbial fauna. Soil samples for microbial community assessment were taken prior to colony establishment within the mesocosms and at the end of the experiments; however, the DNA extracted from these soils were not of a high enough resolution and quantification for analysis (data not presented). Finally, the warming component of our experiment also may have affected lower trophic levels, and other recent experiments have found that ants indirectly can alter decomposition rates by changing the food web structure of soil microarthopods (McGlynn & Poirson 2012).

We consider the effect of ants on soil respiration an example of an indirect ant-mediated ecosystem process (Del Toro, Ribbons & Pelini 2012). In heated mesocosms, soil respiration increased when ants were present, which is consistent with recent work by Jílková & Frouz (2014). Ants may affect soil respiration indirectly in at least two ways. By increasing soil movement, ants can oxygenate soils, changing microbial community structure and increasing respiration rates (DeAngelis *et al.* 2010). In addition, soil movement by ants increases soil porosity and possibly water retention (Cammeraat & Risch 2008), leading to increased respiration when coupled with warming. The well-drained substrate in our mesocosms kept soil moisture low (generally <8%) but even a slight change in soil porosity and water retention can lead to changes in microbial communities and ultimately explain the increased respiration rates observed in our experiment. Soil respiration rates in the presence of *Formica polyctena* were higher in ant nests than in soils with no ants (Jílková & Frouz 2014). In that experiment, increased respiration rates in ant nests may be partially attributable to honeydew inputs that can stimulate microbial communities in the nest soil (Jílková & Frouz 2014). However, the honeydew solution used to feed the ants in our mesocosms was

contained in closed tubes and the solution was only accessible to ants. Ant presence in warmer and forested environments can also lead to increased ant respiration (Jensen & Nielsen 1975; Nielsen 1986; Domisch *et al.* 2006); however, respiration rates in our experiment coming from mesocosms with ants were an order of magnitude higher in these previous studies, indicating that microbial respiration rates are also contributing substantially to total CO_2. Additionally, increases in respiration in the warmer, manual soil manipulation treatments (Fig. 3b) may suggest that there is a strong ant by microbial community interaction that should be further explored in future studies. This is also consistent with Jílková & Frouz (2014) who showed evidence that *Formica polyctena* stimulated microbial respiration.

Ants also indirectly affected nitrogen availability. In ant mesocosms, NH_4^+ and NO_3^- concentrations decreased with temperature relative to empty and manually manipulated soils mesocosms without ants. Zelikova, Sanders & Dunn (2011) found an initial decrease in NH_4^+ availability when *Aphaenogaster* ants were excluded in the field, and Ohashi *et al.* (2007) found that wood ants (in the *Formica rufa* group) increased the availability of nutrients for plants. We also found higher concentrations of nitrogen in mesocosms to which ants were added. Because our study was conducted as an experimental mesocosm manipulation, we were able to limit the effects of potentially confounding factors on the influence of ants on nitrogen availability. These effects are likely to change when ants interact with other invertebrates and indirectly affect microbial communities in more complex field conditions and a variety of soil types (e.g. Zelikova, Sanders & Dunn 2011). Furthermore, the significant warming × soil treatment effect on NH_4^+ that we observed suggests that the role of ants in indirectly mediating nitrogen availability is likely to change as temperature increases. In particular, nitrogen may be less available for plants under future warmer climates if the role of ants on nitrogen availability diminishes.

Collectively, the results from this experiment suggest that in a warming climate the direct and indirect effects of ants on biogeochemical cycles may be disrupted. The direct consequences of warming on ant-mediated processes such as soil movement and decomposition are likely to alter the functional role of ants in the northern temperate zone. Future work should examine the effects of ant-mediated soil movement and decomposition and their interactions with warming on microbial community structure and function in order to better understand the ultimate mechanisms that drive the carbon and nitrogen patterns detected in this experiment. We expect that climatic change will impact eastern North America by increasing precipitation and temperature (IPCC 2013), and the interaction of changing precipitation on ant-mediated ecosystem processes should be a priority for future research. Finally, future studies should consider the impacts of the changes in these ecosystem processes on

productivity and the use and uptake of nutrients made available by ants to primary producers, so that the effects of ants on ecosystem processes can be scaled up.

Acknowledgements

Data accessibility

This data set, R code and detailed analyses outputs are available in Appendix A and are deposited in the Harvard Forest Online data Archive: http://dx.doi.org/10.6073/pasta/5070aeb5acc6cb1ec96778d039efe6bd (Del Toro, Silva & Ellison 2015).

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Datasets and Detailed ANOVA and post-hoc analyses outputs.

Appendix S2. Path analyses datasets and detailed outputs.