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Citation

Del Toro, Israel, Relena R. Ribbons, Aaron M. Ellison. 2015. Ant-mediated ecosystem functions on a warmer planet: effects on soil movement, decomposition and nutrient cycling. *Journal of Animal Ecology* 84 (5): 1233-1241.

Published Version

doi:10.1111/1365-2656.12367

Permanent link

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

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15 **Summary**

- 16 **1.** Direct and indirect consequences of global warming on ecosystem functions and
17 processes mediated by invertebrates remain understudied but are likely to have
18 major impacts on ecosystems in the future. Among animals, invertebrates are
19 taxonomically diverse, responsive to temperature changes, and play major ecological
20 roles which also respond to temperature changes.
- 21 **2.** We used a mesocosm experiment to evaluate impacts of two warming treatments
22 (+3.5 and + 5 °C, set points) and the presence and absence of the ant *Formica*

23 *subsericea* (a major mediator of processes in north-temperate ecosystems) on
24 decomposition rate, soil movement, soil respiration, and nitrogen availability.

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

29 4. In both years, the amount of soil displaced and soil respiration increased in the
30 warming and ant presence treatments (soil movement: 73 – 119%; soil respiration:
31 37 – 48% relative to the control treatments without ants).

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

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

38 **Key words** Climate change, ecosystem processes, *Formica*, soil movement, decomposition,
39 soil respiration, nitrogen availability

40

41 **Introduction**

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

46 direct and indirect roles in major ecosystem processes (Bardgett & Wardle 2010). Field
47 observations and experiments have illustrated that terrestrial invertebrates directly influence
48 decomposition (e.g., shredding of detritus: Wall *et al.* 2008) and soil modification (e.g., burrow
49 construction: Kuiper *et al.* 2013), thus indirectly affecting nutrient cycling and availability
50 (Nielsen *et al.* 2011; Prather *et al.* 2012). These roles may change in magnitude and
51 directionality because of climatic change, potentially leading to changes in ecosystem functions
52 and services (sensu Cardinale *et al.* 2012). However, little is known about the effects of climatic
53 change on invertebrate-mediated ecosystem processes and services (Staudinger *et al.* 2012;
54 Grimm *et al.* 2013). In a recent review Prather *et al.* (2012) suggest that climate change is likely
55 to affect key ecosystem processes and services mediated by invertebrates (e.g. nutrient cycling,
56 decomposition, and habitat formation). Field observations have suggested that ants play major
57 roles as soil bioturbators and can create or modify terrestrial ecosystem structure and function at
58 various scales, but these observations have proven to be difficult to test experimentally (Prather
59 *et al.* 2012). Our study enhances our understanding of ecosystem processes mediated by ants in a
60 novel and simplified experimental design and specifically address the consequences of warming
61 on key ant-mediate ecosystem processes.

62 Among ground-dwelling invertebrates, ants are an ideal study system to use in
63 experiments designed to evaluate impacts of climatic warming on animal-mediated ecosystem
64 processes at the air-soil interface. Ants can directly alter soil movement and decomposition rates,
65 and indirectly affect soil nutrient dynamics (Wardle *et al.* 2011; Del Toro, Ribbons & Pelini
66 2012; Kendrick *et al.* 2015), by their building of nests in the soil, shredding organic material and
67 foraging in leaf litter. Ant activity, abundance, and diversity also respond rapidly to local

68 changes in temperature (Pelini *et al.* 2011). However the additive or interactive effects of
69 warming and ants on ecosystem processes remain largely unexplored.

70 Here, we describe results from a mesocosm experiment designed to assess how warming
71 induces changes in the activity of the ant species *Formica subsericea* Say, 1836 that
72 subsequently alter rates of soil movement, decomposition of leaf litter, soil respiration, and
73 nitrogen availability. We hypothesized that in warmer treatments, increased ant activity would
74 lead to increases in: (1) soil movement; (2) active shredding of leaf litter and its decomposition;
75 (3) soil respiration rate; and (4) nitrogen availability.

76

77 **Materials and methods**

78 *Experimental design*

79 Mesocosm experiments have been used extensively to test hypotheses derived from field
80 observations that cannot be tested with large-scale field experiments because of logistic
81 constraints (Sala *et al.* 2000), and can help simplify complex systems to identify the impact of
82 individual manipulated variables on a constrained number of measured responses. Our
83 mesocosm experiment was a two-way factorial experimental design with two levels of warming
84 (+3.5 °C and +5 °C set-points) crossed with three treatments of soil manipulation: 1) addition of
85 ants (*Formica subsericea*: $N = 96$ [2011]; $N = 82$ [2012]); 2) undisturbed controls ($N = 15$
86 [2011]; $N = 24$ [2012]; and 3) a disturbance control, in which every week, the upper 3-5 cm of
87 the top soil layer was manually overturned the with a trowel and the bottom soil layers were
88 aerated with a 25-cm pick ($N = 12$ [2012 only]). The disturbance control was established to
89 evaluate the effects of manual soil manipulation in comparison to soil manipulation done by ants
90 on the measured response variables. The experimental warming temperatures that we used

91 represented expected summer temperature increases for eastern North America by 2100 under
92 two different climatic change scenarios (i.e. +3.5 °C, RCP6.0 scenario and +5.0 °C, RCP8.5
93 scenario; Deser *et al.* 2012; IPCC 2013). The experiment ran for 30 days in 2011 (June – July)
94 and 85 days in 2012 (May – July). Projected increases in temperatures are likely to reach the
95 critical thermal maxima (Oberg, Del Toro & Pelini 2012) of some common ant species of eastern
96 North America (including *F. subsericea*) that can ultimately have negative impacts on the ant
97 physiology and functionality of ants in temperate ecosystems.

98 Each mesocosm was contained in a 19 L plastic bucket (30 cm diameter and 39 cm
99 height; Fig. 1A). The containers were filled with three layers of soil, which mimicked the soils
100 where *F. subsericea* commonly occurs. The lowest layer consisted of 2.0 L of 1.2-cm sized
101 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
102 top layer consisted of 2.0 L of sand and 2.0 L of heat treated compost. We drilled eight 1-mm
103 diameter holes at the base of each mesocosm to allow excess water to drain out. The top 2 cm of
104 each container was coated with petroleum jelly to prevent the ants from escaping each
105 mesocosm. We cut a 14-cm diameter hole on each of the mesocosm lids and covered the opening
106 with 1-mm fiberglass mesh. The mesh allowed ambient humidity and the heat from the lamps to
107 penetrate the mesocosm, while excluding potential predators and seeds and preventing the ants
108 from leaving the mesocosm. Temperature sensors were placed in the middle of each soil layer,
109 by drilling 5-mm diameter holes and inserting thermocouples into the mesocosm. The holes were
110 then completely sealed with silicone (Fig. 1A). Mean, minimum, and maximum hourly
111 temperatures were recorded on a CR1000 data-logger (Campbell Scientific, Logan, Utah, USA)
112 and downloaded weekly. Humidity was recorded using a CD620 HydroSense water-content
113 sensor (Campbell Scientific) and monitored three times per week to make sure that all treatments

114 remained at a constant soil moisture (5 – 8% soil moisture content). If soil moisture in the
115 mesocosms fell below 5%, they were watered for 5 minutes with a sprinkler irrigation system.
116 Such an extreme reduction in soil moisture occurred only three times in the +5 °C warming
117 treatment and once in the +3.5 °C warming treatment throughout the duration of the experiment.

118 The mesocosms were actively warmed using 250-Watt infra-red heat lamps (Phillips
119 250W, 120 volt heat light) (Fig. 1A). We chose infra-red lighting because we believe this color
120 spectrum would not affect diurnal and nocturnal activity and behaviors in the ants. Previous
121 work with the ant *Formica cuniculaia* (a closely related species of *F. subsericea* in the *fusca*
122 group) shows this species to have a dichromatic visual system and is unable to distinguish color
123 differences at wavelengths >540 nm (Aksoy & Camlitepe 2012) therefore we believe the infrared
124 lighting was not detectable by the ants but the temperature manipulation was. To achieve a mean
125 temperature increase of 3.5 °C, the lamps were hung 120 cm above the surface of the
126 mesocosms. To achieve a mean temperature increase of 5 °C, the lamps were hung 60 cm above
127 the surface of the mesocosms. Lamp bulbs were left on continuously so that daily temperature
128 fluctuations were consistent across the treatments; bulbs were replaced as needed. One lamp
129 evenly heated four mesocosms through all soil layers, so we clustered the mesocosms in groups
130 of four; the distribution of the soil manipulation treatments was randomized within each
131 temperature treatment. Actual hourly mean temperatures experienced within each mesocosm in
132 the “+3.5 °C treatment” ranged from 0.5 – 3.9 °C warmer than controls, whereas the “+5 °C
133 treatment” ranged from 1.7 – 6.7 °C warmer than controls (Fig. 1B and 1C), with the largest
134 temperature differences occurring during the coolest and warmest times of the day. These soil
135 temperatures are consistent with air and soil temperature variation observed at another Harvard
136 Forest field site where open-top chambers are being warmed (Pelini *et al.* 2011) to replicate

137 IPCC warming projections and at the Fisher meteorological station in Harvard Forest (Fig. 1B
138 and 1C, grey line) which documents daily temperature variation.

139 *The ant*

140 *Formica subsericea* is a common and widely distributed species that builds large nests in
141 soil. Its large colonies (hundreds to > 10,000 workers) typically are found in edge and open
142 habitats throughout eastern North America (Ellison *et al.* 2012). The genus *Formica* has a
143 Holarctic distribution and many of the species in the diverse and widespread *fusca* group (which
144 includes *F. subsericea*) share similar natural history traits (Francoeur 1973; Gösswald 1989). We
145 expect, therefore, that the results from our experiment are likely to apply to other north
146 temperate-zone soils where *Formica fusca*-group species occur. We collected ant colonies from
147 sandy soils at three localities in central Massachusetts: the Montague Pine Barrens (42.569 °N, -
148 72.536 °W), the Devens Pine Barrens (42.452 °N, -71.641 °W), and Myles Standish State Forest
149 (41.839 °N, -70.691 °W). Each mesocosm was established with 100 workers from independent
150 ant colonies; no queens were collected or used in the mesocosms. We expected that individual
151 activity and behavior would not be affected by the absence of queens as ants self-organize tasks
152 without the need of direction from a reproductive caste (Oster & Wilson 1978; Gordon 2010).

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

160 *Ecosystem processes*

161 We measured mass loss (as an indication of decomposition rate) by placing two
162 fiberglass mesh bags filled with leaf litter on top of the soil in each mesocosm. Each bag
163 contained 1 g of dried (70 °C for 72 hr) whole red maple (*Acer rubrum* L.) leaves + 1 g of dried
164 whole red oak (*Quercus rubra* L.) leaves. One litter bag excluded ants because of the size of the
165 mesh (1-mm), while the second bag allowed ants access to the litter (mesh size = 10-mm). At the
166 end of the experiment, litter bags were removed from the mesocosms; the remaining litter was
167 dried (70 °C for 72 hr) and weighed (± 0.001 g), to determine mass lost. At the end of the
168 experiment, the 10-mm mesh bags contained much smaller portions of leaves and often had ants
169 still inside the bags when opened (personal observation), whereas no ants were ever detected in
170 the 1-mm mesh bags.

171 Soil respiration (as CO₂ flux) was measured for 5 min every 7 days in 2011 and every 14
172 days in 2012 using a portable infra-red gas analyzer (LI-COR 6400, LI-COR Biosciences,
173 Lincoln, Nebraska) with a soil-flux chamber (LI-COR 6400-09) placed on a PVC collar (20-cm
174 diameter, 5-cm deep) half-buried at the soil surface in a subset of the mesocosms ($N = 38$ [2011];
175 $N = 72$ [2012]). We used linear interpolation and integration (based on the relationship between
176 measured soil temperature and CO₂ flux; Savage, Davidson & Tang 2013) to estimate the
177 amount of CO₂ produced per day over the course of the experiment each year.

178 We determined the total amount of available nitrogen (NH₄⁺ and NO₃⁻) as that captured
179 on ion-exchange resin bags placed in each mesocosm (Maynard, Kalra & Crumbaugh 2008).
180 Approximately 5 g of resin was placed in a nylon mesh bag and pretreated with 2 mol L⁻¹ KCl
181 before being placed in the mesocosms. Resin bags were placed 3 cm below the surface of the soil
182 and left in the mesocosm for the duration of the experiment during the 2012 season (Fig. 1A).

183 Resins were returned to the laboratory and dried at 105°C for 24 hours. Resins were then
184 extracted in 100 mL of 1 mol L⁻¹ KCl for 48 hours. Resin extracts were filtered through a coarse
185 pore filter (0.45-0.60 µm), and inorganic N concentrations were determined colorimetrically with
186 a Lachat AE flow-injection auto-analyzer (Lachat Instruments, Inc., Loveland, Colorado, USA)
187 using the indophenol-blue and cadmium reduction methods for NH₄, and NO₃ respectively.
188 Nitrogen mineralization is a reliable predictor of soil productivity (Raison, Connell & Khanna
189 1987), and total nitrogen captured on resins is closely correlated with nitrogen mineralization
190 (Strader, Binkley & Wells 1989). Net nitrogen capture was determined by subtracting the
191 concentration of inorganic nitrogen (NH₄⁺ + NO₃⁻) in the incubated samples from that of the
192 initial samples (Morecroft, Marrs & Woodward 1992). High net nitrogen capture can be
193 indicative of high levels of microbial activity in the soil (Chapin, Matson & Mooney 2002).

194 We estimated the amount of soil moved by *F. subsericea* by sacrificing the colony at the
195 end of the experiment and creating a three-dimensional paraffin cast of the nest (Gulf Wax®,
196 Royal Oak, Roswell, GA) as described by Tschinkle (2010). We estimated the volume of soil
197 displaced from the weight of the cast and the density of the paraffin (0.9 g/cm³). We also
198 recorded the maximum depth (cm from the surface) for every cast.

199

200 *Data analysis*

201 We used Analysis of Variance (ANOVA) and post-hoc Tukey pairwise comparisons to
202 test the effects of warming and different forms of soil alteration (predictor variables) on the
203 direct and indirect ecosystem processes of soil movement, decomposition rate, soil respiration,
204 and nitrogen availability (response variables) (R code, datasets and detailed ANOVA outputs are
205 in Appendix A). We divided the processes into two categories, those directly mediated by ants

206 and indirect effects expressed as the ant \times treatment interaction. We defined direct processes as
207 those where the ants came in direct contact with the substrate (e.g., soil moved or litter shredded)
208 and indirect processes as those where the consequence of a direct effect altered the process in
209 question (e.g., soil respiration is influenced by soil moved and nitrogen capture is influenced by
210 shredded or decomposed litter).

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

217

218 **Results**

219 *Direct effects of ants on ecosystem processes*

220 In 2011, soil-surface temperatures ranged from 11 – 31 °C in the control treatment,
221 whereas the soil surface in the warmest treatment reached a maximum of 38 °C. In 2012, soil-
222 surface temperatures ranged from 5 – 32 °C in the controls and reached a maximum of 40 °C in
223 the high temperature treatment (Figs. 1B, 1C). In both 2011 and 2012, ants displaced
224 significantly more soil ($P < 0.001$; Figs. 2A, 2B) and built deeper nests in the warmer treatments
225 than in the controls ($\bar{x} = 15.5 \pm 0.8$ [SD] cm in the controls; 22 ± 1.2 cm in the +3.5 °C treatment;
226 23 ± 1.2 cm in the +5 °C treatment, $P < 0.01$ [2011]; and $P = 0.03$ [2012]). Ants actively foraged
227 inside the 10-mm decomposition bags, shredding leaf litter and carrying leaf particles back to the
228 nest, but we did not observe similar foraging in the 1-mm mesh bags. Decomposition was higher

229 in the 10-mm mesh bags than the 1-mm mesh bags (44% more litter loss in 2011, and 61% more
230 litter loss in 2012; $P < 0.001$), and litter loss decreased with increasing temperature (Figs. 2C,
231 2D; 84% decrease, $P < 0.0001$ [2011]; 52% decrease, $P = 0.002$ [2012]). In 2011 there was a
232 significant ant \times warming interaction in leaf-litter-mass lost, with less litter lost in the warming
233 treatments (Fig. 2C). In 2012 this interaction was not significant, but the trend was in the same
234 direction (Fig. 2D, $P = 0.09$). Decomposition was highest in the controls in 2012, and there were
235 no significant differences among the ant addition or undisturbed warming treatments (Fig. 2D).

236

237 *Indirect effects of ants on ecosystem processes*

238 In both 2011 and 2012, the presence of ants was associated with an $\approx 25\%$ increase in soil
239 respiration rate ($P < 0.01$ [2011]; $P < 0.05$ [2012]; Figs. 2A, 2B). In contrast, we observed an
240 effect of warming on soil respiration rate only in 2012: soil respiration rate was 38% higher in
241 the +3.5 °C treatment and 28% higher in the +5 °C warming treatment than in the control ($P =$
242 0.30 [2011]; $P < 0.01$ [2012]; Figs. 2A, 2B). In 2011 there also was a significant ant \times warming
243 interaction (Fig. 3A), but this interaction was not observed in 2012 (Fig. 3B).

244 Ammonium (NH_4^+) concentration in mesocosms with ants decreased 74% as temperature
245 increased ($P < 0.01$, Fig. 3C), but there were no effects of temperature on NH_4^+ concentrations in
246 the empty mesocosm controls. There was a significant soil treatment \times warming interaction: as
247 NH_4^+ concentrations in the ant mesocosms decreased in the warmer treatments, NH_4^+
248 concentrations in controls increased ($P < 0.01$, Fig. 3C).

249 Similarly, when ants were present, nitrates (NO_3^-) tended to decrease to undetectable
250 amounts with warming ($P = 0.09$, Fig. 3D). Although NO_3^- concentrations were highest when

251 ants were present in unheated mesocosms ($P = 0.04$; Fig. 3D), there were no differences in NO_3^-
252 concentrations among mesocosms heated to either +3.5 or +5.0 °C.

253 Effects of ants, warming, and their interaction on NH_4^+ and NO_3^- resulted in significant
254 effects on total available nitrogen ($P < 0.01$, Appendix A). In particular, when ants were added to
255 unheated mesocosms, nitrogen capture (a combination of mineralization and total nitrogen) was
256 four times higher than in empty mesocosms and the disturbance controls. These differences
257 were much less pronounced in warmed mesocosms and were driven largely by NH_4^+
258 concentrations.

259

260 *Path analyses*

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

269

270 **Discussion**

271 Global climatic changes are likely to impact ecosystem processes and services mediated
272 by arthropods, but the cascading and interacting effects of climate change on arthropods and
273 ecosystem functions remain understudied. Our experimental results in mesocosms support

274 conclusions from field observations that ants can influence soil movement and soil respiration, at
275 both current and projected temperatures (Domisch *et al.* 2006). However, the effects of ants on
276 decomposition and nitrogen availability that are seen at current temperatures (e.g., Del Toro *et*
277 *al.* 2012; Prather *et al.* 2012; Kendrick *et al.* 2015) are much reduced in warmer temperatures. As
278 ants reach temperatures close to their critical thermal maximum, ant functional roles may change
279 in response to thermal stress. We speculate that the reductions in decomposition and nitrogen
280 availability could be due to reduced ant surface activity, but this merits further investigation.
281 Because we used mesocosm experiments to simplify the interactions between ants and ecosystem
282 function, our findings may not be entirely representative of natural systems, but rather show the
283 potential influence of warming on processes mediated by ants.

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

295 Ants decomposed more leaf litter under ambient temperatures, and up to 84% less in
296 warmer treatments. In fact, litter decomposition rates in the +5 °C mesocosms with ants were not

297 different from litter decomposition rates in mesocosms without ants. Decreased decomposition in
298 the warmed treatments may be attributable in part to desiccation caused by the infra-red heat
299 lamps, but the significant warming \times soil treatment term also may indicate that ants reduced their
300 surface activity in the warming treatments (see also Diamond *et al.* 2013), resulting in less litter
301 shredding. Stuble *et al.* (2014) demonstrated that within experimental warming chambers at
302 Harvard Forest and Duke Forest (which have similar temperature increases as our experiment)
303 one metric of ant activity, foraging, was not influenced by temperature increases. We suggest
304 that ants directly affect decomposition by less actively shredding leaf litter and that ant-mediated
305 decomposition rates may decline further under future warming conditions. This pattern may
306 apply to other species in the genus *Formica* as they often use leaf litter material in their nest
307 construction (Gösswald 1989; Kadochová & Frouz 2013).

308 In contrast, Wardle *et al.* (2011) observed that decomposition rate increased when ants
309 were excluded. Our results could have differed from those presented in Wardle *et al.* (2011)
310 because of unmeasured differences in soil quality and microbes. The sandy soils we used
311 typically have low water retention and osmotic potential (Chowdhury, Marschner & Burns 2011)
312 and species-poor microbial communities. The soil strata built in these mesocosms were meant to
313 reflect similar soil conditions to those found in naturally occurring *F. subsericea* nests, however
314 it is likely that our soils are not a perfect replicate of field nest conditions due to the sterilization
315 and soil disturbances necessary to build the mesocosms. Wardle *et al.* (2011) completed their
316 work in the field, with moister forest soils that support a much richer soil microbial fauna. Soil
317 samples for microbial community assessment were taken prior to colony establishment within
318 the mesocosms and at the end of the experiments, however the DNA extracted from these soils
319 were not of a high enough resolution and quantification for analysis (data not presented). Finally,

320 the warming component of our experiment also may have affected lower trophic levels, and
321 other recent experiments have found that ants indirectly can alter decomposition rates by
322 changing the food-web structure of soil microarthropods (McGlynn & Poirson 2012).

323 We consider the effect of ants on soil respiration an example of an indirect ant-mediated
324 ecosystem process (Del Toro *et al.* 2012). In heated mesocosms, soil respiration increased when
325 ants were present, which is consistent with recent work by Jílková & Frouz (2014). Ants may
326 affect soil respiration indirectly in at least two ways. By increasing soil movement, ants can
327 oxygenate soils, changing microbial community structure and increasing respiration rates
328 (DeAngelis *et al.* 2010). In addition, soil movement by ants increases soil porosity and possibly
329 water retention (Cammeraat & Risch 2008), leading to increased respiration when coupled with
330 warming. The well-drained substrate in our mesocosms kept soil moisture low (generally <8%)
331 but even a slight change in soil porosity and water retention can lead to changes in microbial
332 communities and ultimately explain the increased respiration rates observed in our experiment.
333 Soil respiration rates in the presence of *Formica polyctena* were higher in ant nests than in soils
334 with no ants (Jílková & Frouz 2014). In that experiment, increased respiration rates in ant nests
335 may be partially attributable to honeydew inputs that can stimulate microbial communities in the
336 nest soil (Jílková & Frouz 2014). However, the honeydew solution used to feed the ants in our
337 mesocosms was contained in closed tubes and the solution was only accessible to ants. Ant
338 presence in warmer and forested environments can also lead to increased ant respiration (Jensen
339 & Nielsen 1975; Nielsen 1986; Domisch *et al.* 2006) however respiration rates in our experiment
340 coming from mesocosms with ants were an order of magnitude higher in these previous studies,
341 indicating that microbial respiration rates are also contributing substantially to total CO₂.
342 Additionally increases in respiration in the warmer, manual soil manipulation treatments (Fig.

343 3B) may suggest that there is a strong ant by microbial community interaction that should be
344 further explored in future studies. This is also consistent with Jílková & Frouz (2014) who
345 showed evidence that *Formica polyctena* stimulated microbial respiration.

346 Ants also indirectly affected nitrogen availability. In ant mesocosms, NH_4^+ and NO_3^-
347 concentrations decreased with temperature relative to empty and manually manipulated soils
348 mesocosms without ants. Zelikova *et al.* (2011) found an initial decrease in NH_4^+ availability
349 when *Aphaenogaster* ants were excluded in the field, and Ohashi *et al.* (2007) found that wood
350 ants (in the *Formica rufa* group) increased the availability of nutrients for plants. We also found
351 higher concentrations of nitrogen in mesocosms to which ants were added. Because our study
352 was conducted as an experimental mesocosm manipulation, we were able to limit the effects of
353 potentially confounding factors on the influence of ants on nitrogen availability. These effects
354 are likely to change when ants interact with other invertebrates and indirectly affect microbial
355 communities in more complex field conditions and a variety of soil types (e.g. Zelikova *et al.*
356 2011). Furthermore, the significant warming \times soil treatment effect on NH_4^+ that we observed
357 suggests that the role of ants in indirectly mediating nitrogen availability is likely to change as
358 temperature increases. In particular, nitrogen may be less available for plants under future
359 warmer climates if the role of ants on nitrogen availability diminishes.

360 Collectively, the results from this experiment suggest that in a warming climate the direct
361 and indirect effects of ants on biogeochemical cycles may be disrupted. The direct consequences
362 of warming on ant-mediated processes such as soil movement and decomposition are likely to
363 alter the functional role of ants in the northern temperate zone. Future work should examine the
364 effects of ant-mediated soil movement and decomposition and their interactions with warming on
365 microbial community structure and function in order to better understand the ultimate

366 mechanisms that drive the carbon and nitrogen patterns detected in this experiment. We expect
367 that climatic change will impact eastern North America by increasing precipitation and
368 temperature (IPCC 2013), and the interaction of changing precipitation on ant-mediated
369 ecosystem processes should be a priority for future research. Finally, future studies should
370 consider the impacts of the changes in these ecosystem processes on productivity and the use and
371 uptake of nutrients made available by ants to primary producers, so that the effects of ants on
372 ecosystem processes can be scaled up.

373

374 **Acknowledgements**

375 This research was supported by the Harvard Forest NSF LTER (DBI 10-03938), the US
376 DOE PER (DE-FG02-08ER64510 to R. Dunn, A. Ellison, N. Gotelli, and N. Sanders), UMASS
377 Amherst OEB Research Grant (awarded to I. Del Toro), and a NEAGEP fellowship (awarded to
378 I. Del Toro). We thank J. Tang, N. Manyak, K. Davis, and M. Combs for technical advice and
379 support, and N. Gotelli, S. Pelini and N. Sanders for constructive comments on early versions of
380 the manuscript.

381 **Data Accessibility**

382 This dataset, R code and detailed analyses outputs are available in Appendix A and will be
383 deposited in the Harvard Forest Online data Archive < [http://harvardforest.fas.harvard.edu/data-](http://harvardforest.fas.harvard.edu/data-archive)
384 [archive](http://harvardforest.fas.harvard.edu/data-archive)>.

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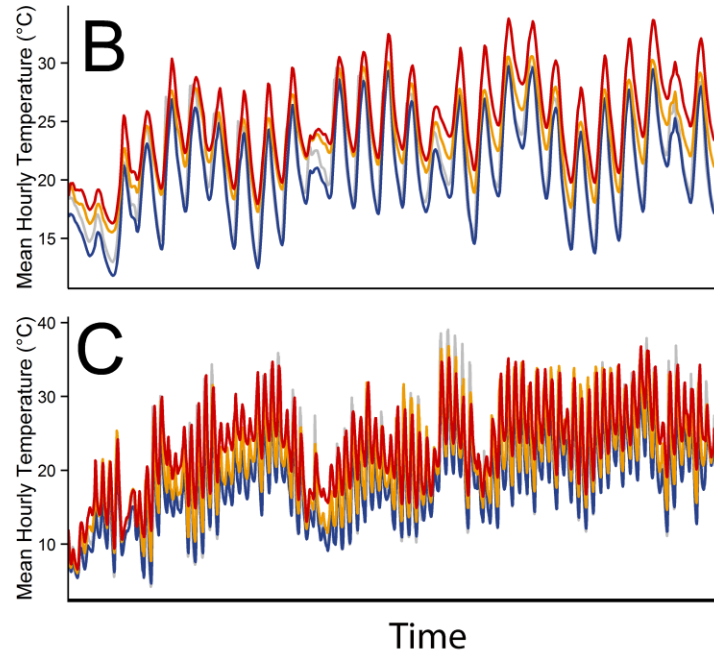
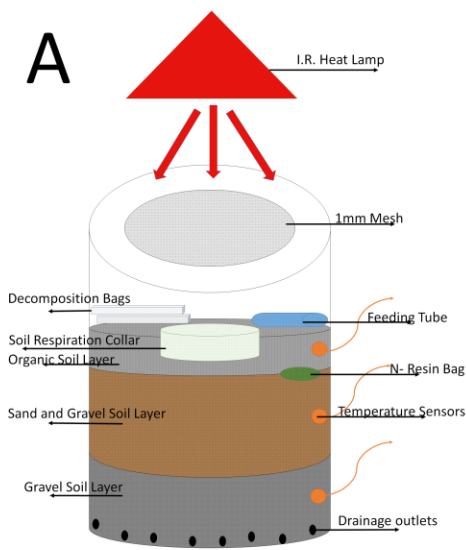
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521 **Figures**



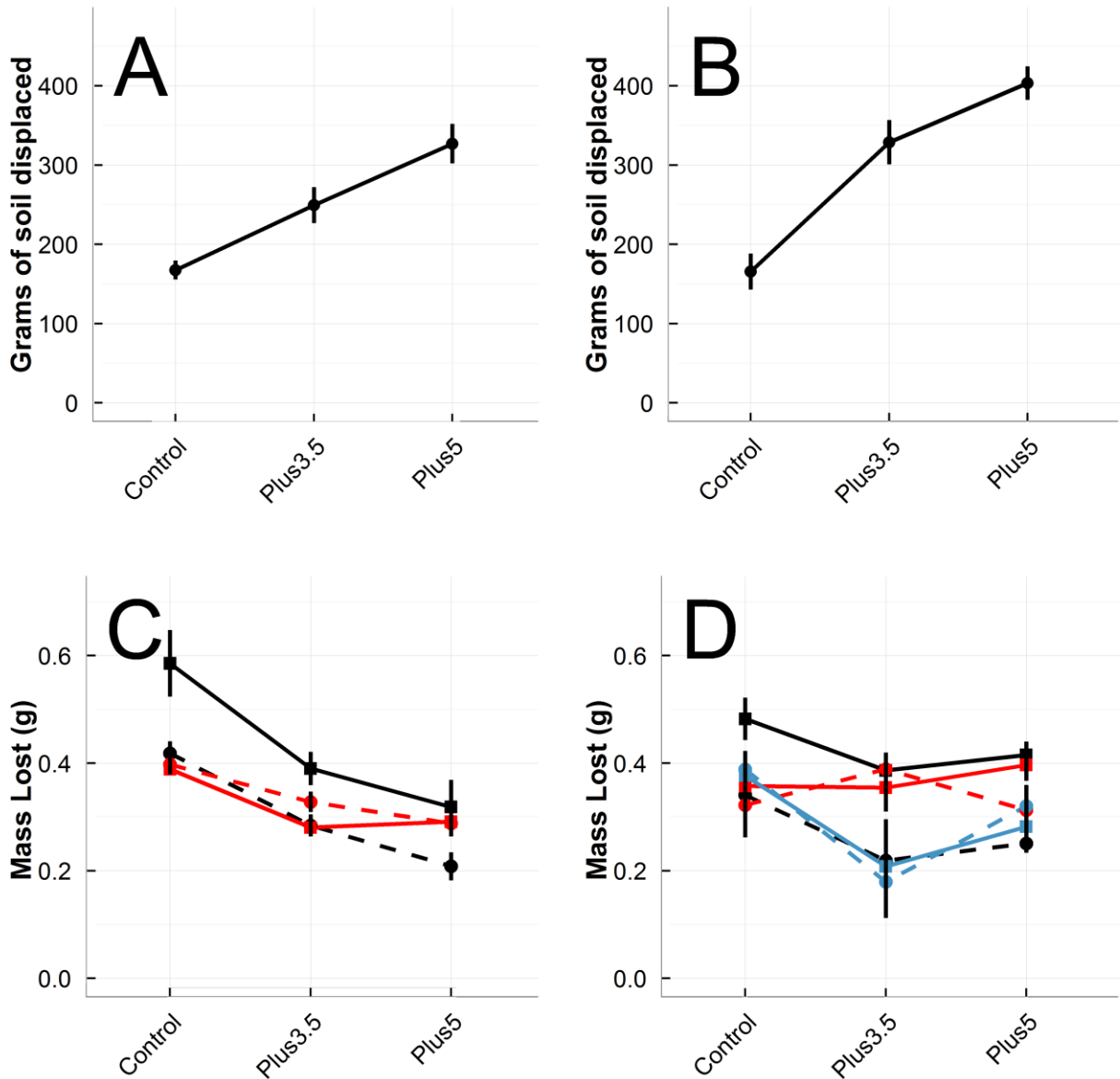
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523 Figure 1: Diagram of the mesocosm design and temperature variation in the three experimental
 524 warming treatments. A) Mesocosm diagram B) Mesocosm temperature variation in 2011 season.

525 Blue lines show the mean temperatures for the control treatment, orange lines show the mean
 526 temperatures for the +3.5 warming treatment and red lines show the mean temperatures for the
 527 +5 treatment. Grey lines show the temperature data from the Harvard Forest Fisher

528 Meteorological Station for the same dates. C) Mesocosm temperature variation in 2011 season,
 529 colored lines are the same as in panel B.

530



531

532 Figure 2: Interaction plots (as in Gotelli & Ellison 2013, chapter 10) of the direct effects of ants

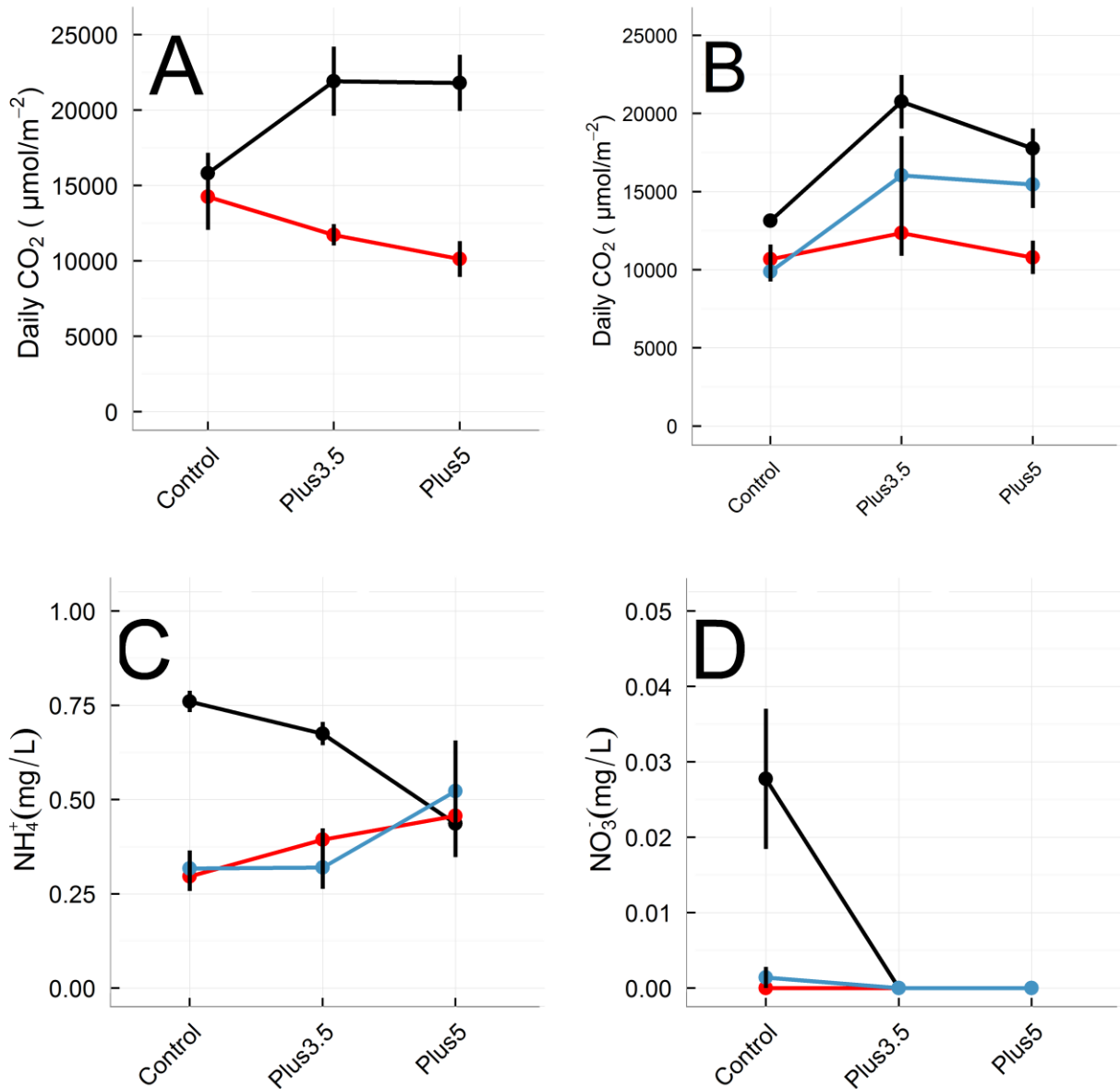
533 on soil movement (A, B) and decomposition (C, D) in 2011 (A,C) and 2012 (B,D). Values

534 shown are means ± 1 standard error of the mean. Line and symbol colors indicate ant additions

535 (black); disturbance controls (blue); and undisturbed controls (red). In C and D, solid lines

536 denote the 10-mm-mesh decomposition bags (ants had access to the litter) and dashed lines

537 denote the 1-mm-mesh decomposition bags (ants did not have access to the litter).



538

539 Figure 3: Interaction plots of the indirect effects of experimental treatments on soil respiration

540 (A: 2011; B: 2012) and nitrogen availability (C: NH₄ in 2012; D: NO₃ in 2012). Line and symbol

541 colors indicate ant additions (black); disturbance controls (blue); and undisturbed controls (red).

542 Figure 4: Path analysis of warming and ant effects on ecosystem processes in 2011 (A) and 2012

543 (B). Each box represents a measured predictor or response variable. Arrows show the

544 hypothesized cause and effect relationships between the variables, and line width is scaled by the

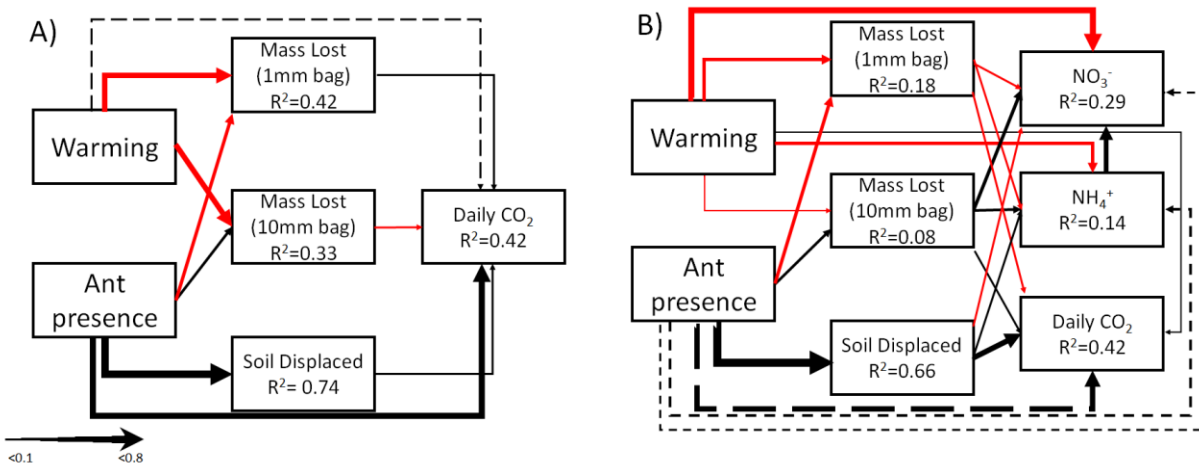
545 loading coefficients. Red lines indicate negative relationships, and black lines indicate positive
 546 relationships. Dashed lines indicate relationships where indirect effects were greater than direct
 547 effects. Coefficients of determination (R^2) are reported in boxes with response variables

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

560