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

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*

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

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 specficially 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

4

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   lightling 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 ($\pm$ 0.001g), 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 CO_2 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 CO_2 flux; Savage, Davidson & Tang 2013) to estimate the

177 amount of CO_2 produced per day over the course of the experiment each year.

178   We determined the total amount of available nitrogen (NH_4^+ and NO_3^-) 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 NH_4, and NO_3 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 ($NH_4^+ + NO_3^-$) 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/cm^3). 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 × 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 × 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 ≈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 × 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 × 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 × 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

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 × 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 microarthopods (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 CO_2.

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 × 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

16

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

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-

384 archive>.

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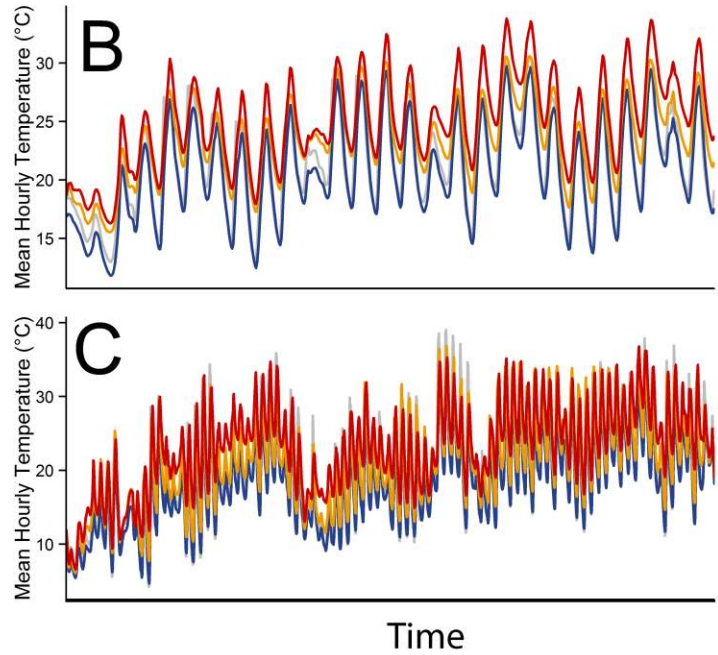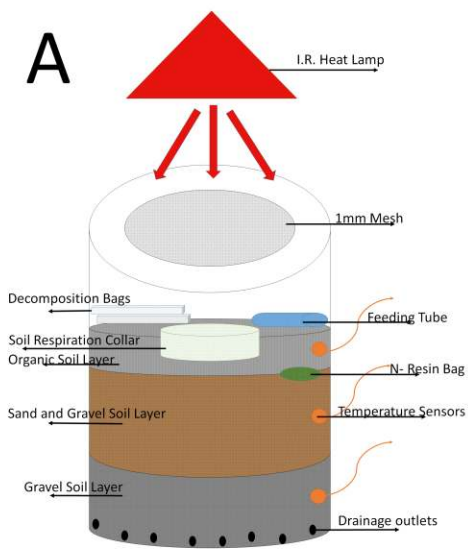
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521 **Figures**

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Figure 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,

colored lines are the same as in panel B.

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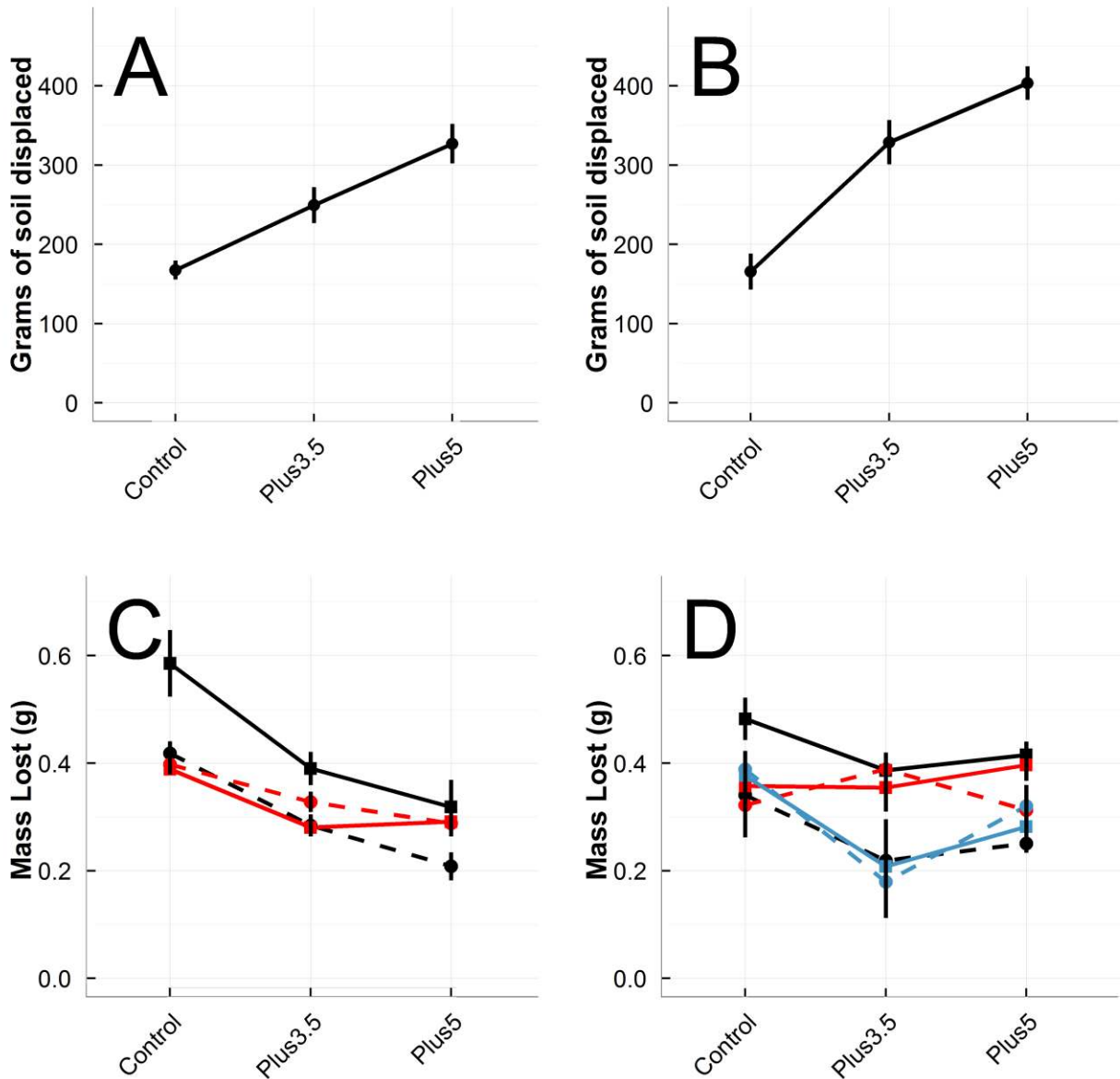
Figure 2: Interaction plots (as in Gotelli & Ellison 2013, chapter 10) of the direct effects of ants

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).

538
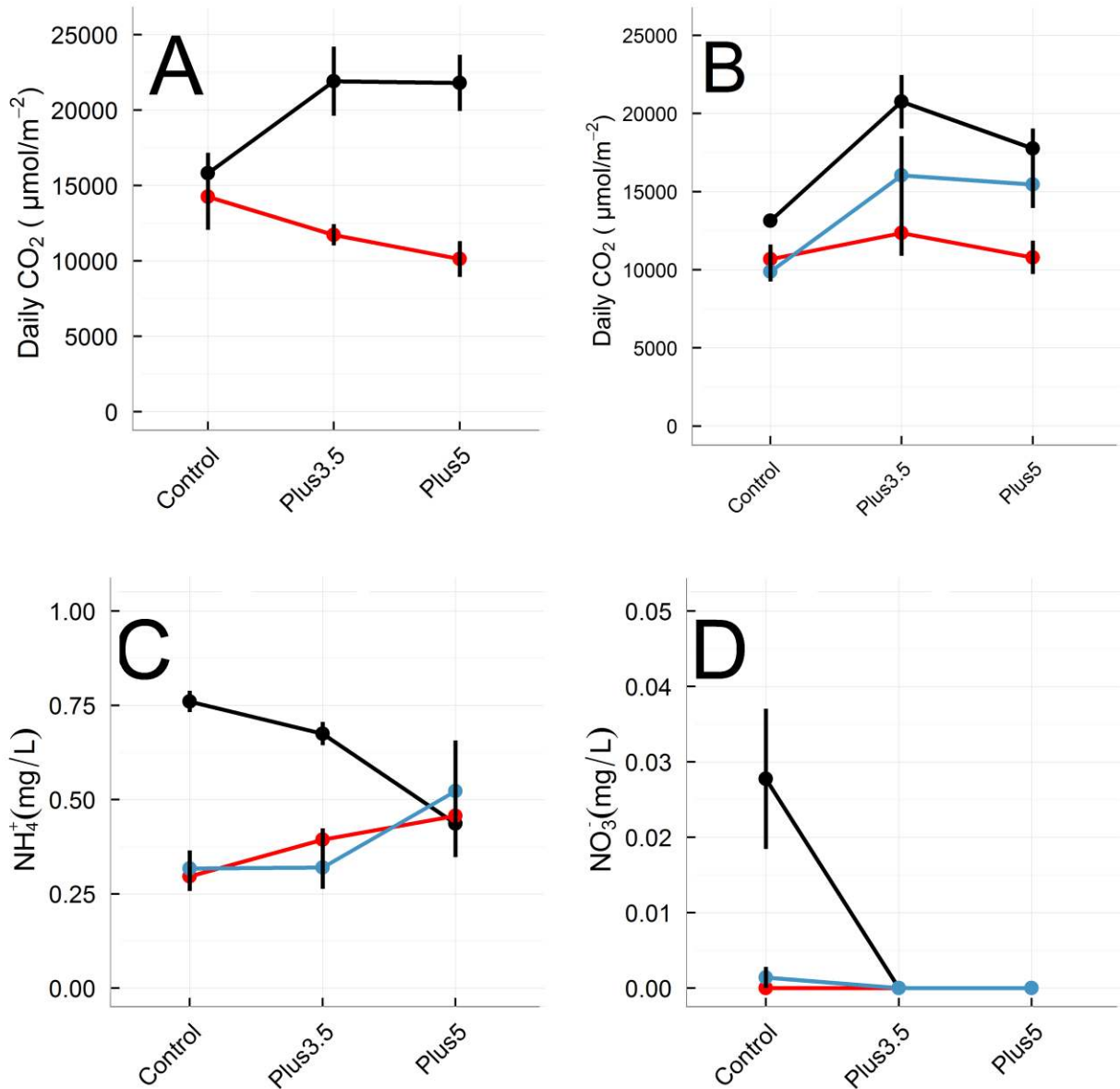
Figure 3: Interaction plots of the indirect effects of experimental treatments on soil respiration

(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).

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

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

hypothesized cause and effect relationships between the variables, and line width is scaled by the
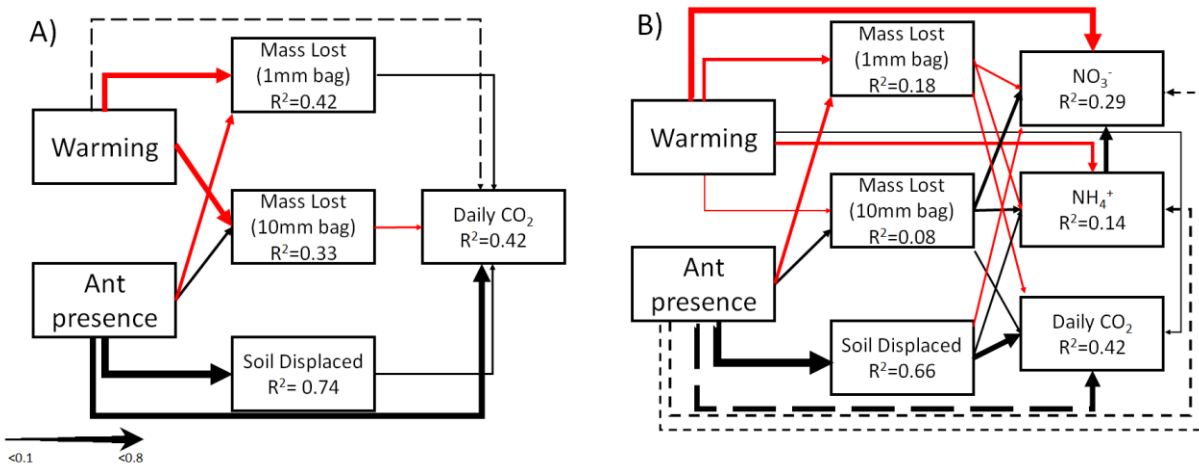
545 loading coefficients. Red lines indicate negative relationships, and black lines indicate positive

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.

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