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Short communication

The effects of amoebal bacterivory on carbon and nitrogen dynamics depend on temperature and soil structure interactions

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ABSTRACT

Fundamental questions remain about the role of bacterivorous protists in regulating soil carbon (C) and nitrogen (N) cycling, including the ways in which protists interact with physical and chemical factors to influence soil decomposer responses to increased temperature. Amoebae in particular deserve attention given their status as one of the most abundant soil protozoans. Using microcosms of simplified soil communities, we investigated the net and interactive effects of amoebal bacterivory, soil aggregate structure, agricultural management (till vs. no-till), and temperature on C and N dynamics during a 24 day-incubation. The respiration temperature sensitivity (Q_{10}) was much more variable for the simplified communities than natural communities, illustrating the challenge in using low-diversity systems to predict such a complex and emergent property. In addition to the expected positive effect of amoebal predation on C and N mineralization in all treatments, we found that the magnitude of this effect was significantly influenced by aggregate structure and temperature. Statistically higher ($P < 0.01$) predation-induced C mineralization in crushed aggregates relative to intact aggregates was observed at 25 °C but not at 15 °C, implying that (i) amoebal predation efficiency is more limited by physical accessibility under higher temperature and/or (ii) a temperature-induced shift in predator species with differing trophic niches. Our results show the importance of better understanding the interactions of the soil food web, aggregate structure, and temperature when predicting soil C and N dynamics under warming scenarios.

A key component of soils is the assemblage of organisms present, members of which are responsible for carrying out many small scale processes that underlie important biogeochemical functions (Urich et al., 2008). Protozoan predation on bacteria has been shown to be an important factor affecting soil nutrient (C and N) turnover rates (Coleman et al., 1977; Stout, 1980; Frey et al., 1985), but the specific effects of physical and environmental factors on this relationship remain understudied. Amoebae are one of the most abundant groups of protists in soils (Ekelund and Rønn, 1994), and although they have been proposed to be less sensitive to soil structure variation due to their unique trophic morphology (Elliott et al., 1980), little has been done to examine the extent to which amoebal predation effects change with aggregate structure, especially under climate change scenarios. Here we thus investigated what effects soil management practices and aggregate structure have on the ability of amoebae to influence soil biogeochemical responses to warming.

Two well-characterized adjacent allophanic Andisols (Table 1) were selected from experimental fields in Tsukuba, Japan (36.024045° N, 140.111558° E) with a mean annual air temperature of 13.7 °C and rainfall of 1300 mm yr^{-1}. One soil received annual tilling (till) and the other received no tilling, but an addition of green manure each year (no-till). More site and soil characteristics have been described elsewhere (Wagai et al., 2013). Each soil was sieved on site to retain aggregates between 4 mm and 8 mm and then air dried. Plant detritus was manually removed and half of the no-till aggregates were moderately crushed by motor and pestle to assess physical structure effect. The three soil treatments (Till [T], Intact no-till [NT], Crushed no-till [NTC]) (Supplemental Fig. 1) were then sterilized using ≥36 kGy of gamma radiation. Simple bacterial communities for re-inoculation were obtained by culturing *Escherichia coli* (ATCC #47076) and *Klebsiella pneumoniae* (ATCC #13882) on weak malt-yeast agar (Shadwick et al., 2009), which were then centrifugally washed (10,000 RCF for 10 min) three times in Page's Amoeba Saline (PAS) (Page, 1988). These

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Table 1
Soil Characteristics. Total C and N content, C to N ratio, and pH of each soil. Soils were sampled in May 2013 from long-term experimental plots in the experimental agricultural field at the National Institute for Agro-Environmental Science, Ibaraki, Japan. The no-till plot has been under no-till management for 28 years, including annual addition of green manure at roughly 7 ton C ha^{-1}. The till plot has been under conventional tillage practice.

Soil (0–5 cm)	%C	%N	C:N	pH
Till	5.20	0.42	12.40	6.16
No-till	14.2	0.99	14.3	6.10

strains were previously shown in a pilot study to grow effectively in both soils and at both temperatures.

Rosenburg et al. (2009) showed that an amoeba (*Acanthamoeba castellanii*) had a strong influence on rhizosphere bacterial communities, particularly on *Betaproteobacteria* and *Actinobacteria*, as opposed to the *Gammaproteobacteria* (which we used here), but if this same pattern held true for the amoebal strains used in the current study it would only serve to attenuate any observed effects of bacterivory. Further, the ability of our bacterial strains to persist and respire in the soil, along with our ability to grow our amoebal strains on them suggests that they were appropriate for this system. Still, further work should consider whether other bacterial strains yield similar results.

Amoeboid predators were obtained by culturing *Dictyostelium discoideum* (strain V12, NBRP, www.nbrp.jp), *Acanthamoeba polyphaga* (ATCC #50372 – originally isolated from Japan) and *Endostelium zonatum* (cultured from the no-till soil *in situ*; identified morphologically using Spiegel et al. (2007); axenized from spores onto heat-killed *E. coli*) on weak malt-yeast agar with the same bacterial inoculum *E. coli* strain (ATCC #47076) as a food source. *D. discoideum* and *A. polyphaga* were originally obtained as axenic cultures and thus did not pose a risk of unwanted bacterial contamination, however, the culture of *E. zonatum* was obtained from the local soil. It is difficult to remove all concomitant bacteria from natural amoeba isolates and though no bacterial endosymbionts are known from *Endostelium* it is theoretically possible that some contaminants were not removed during axenization. No bacterial growth was observed near axenized *Endostelium* isolates prior to culture with *E. coli* and, barring any undetected contamination, each microcosm treatment received an equivalent inoculum of only the two desired species of bacteria. Cultured amoebae were centrifugally washed (at 500 RCF for 10 min) three times in PAS to remove as many *E. coli* cells as possible and all resultant cells in suspension were quantified visually using a hemocytometer. At the conclusion of incubations, cultures of soil suspensions were evaluated by bacterial colony morphology on soil agar and only *K. pneumonia* and *E. coli* were observed.

The experiment was factorially designed to investigate the interaction of temperature, soil type, and predation, such that there were 5 replicate microcosms for each combination of factor levels, and 3 replicates of sterile and unsterilized (natural soil community) controls for each treatment. For each experimental unit, 3 g of each sterilized soil was carefully mixed with 12 g of fully-combusted sand inside 50 mL septum-sealed microcosm jars under aseptic conditions. Bacterial inoculum (to equal 2.0×10^7 mixed cells $\cdot$ g^{-1} dry soil) was added to all jars except natural community and sterile controls, which received equivalent amounts of sterile PAS, and all jars were incubated in the dark at 15 °C for four days to allow bacteria to colonize the substrate (Altenburger et al., 2010). At the end of this initial incubation, soils were brought to 60% water holding capacity (Till: 0.22 ml g^{-1}; Intact no-till: 0.25 ml g^{-1}; Crushed no-till: 0.26 ml g^{-1}) using either live amoebal inoculum (to equal 2.9×10^5 cells g^{-1} dry soil) or an equivalent amount of autoclaved amoebal inoculum. Initial CO_2 readings were immediately made using a Li-Cor 7000 Infrared Gas Analyzer (Li-Cor, Lincoln, Nebraska, USA). Half of the jars were then moved to incubate in the dark at 25 °C. Subsequent headspace gas measurements were made at 3- to 5-day intervals for 24 days.

The presence of amoebae significantly increased the amount of cumulative C respired in all treatments (Fig. 1) ($P \leq 0.01$ for all treatments at day 24; General linear model ANOVA). These results

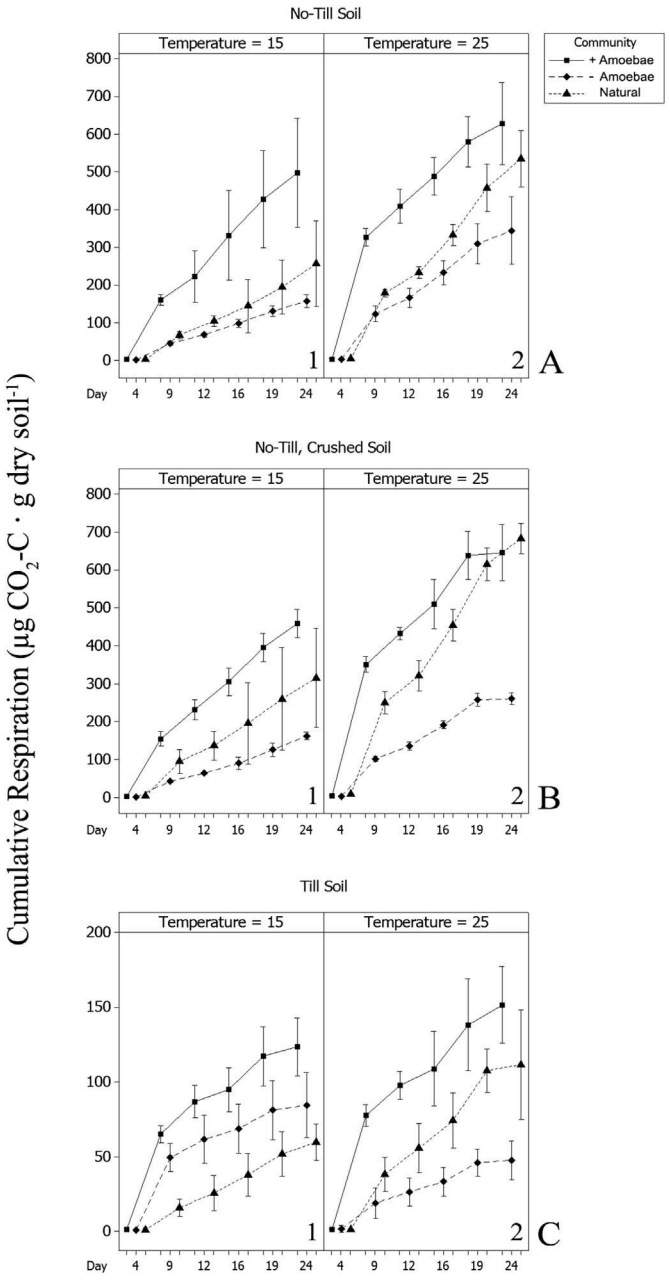

Fig. 1. Cumulative respiration throughout incubations. Cumulative respiration (µg CO_2–C · g dry $soil^{-1}$) for each treatment group (sterile controls not included), with soil treatments separated into three main panels: A = Intact no-till soil, B=Crushed no-till soil, C=Tilled soil; Temperature treatments as sub-panels: 1 = 15 °C, 2 = 25 °C; Community inoculum treatments: Square = artificial community + predators (amoebae), Diamond = artificial community with no predators (bacteria only), Triangle = natural community controls. Error bars denote 95% confidence intervals for the mean. Note y-axis scale in panel C.

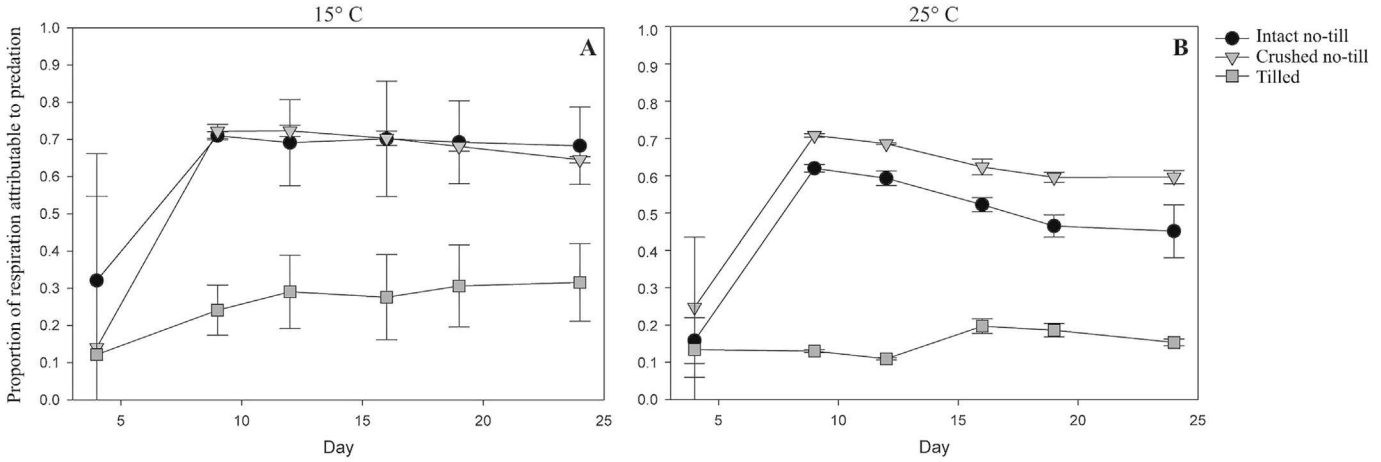

Fig. 2. Proportion of respiration attributable to predation. The proportionate increase in respiration due to the introduction of predators (the difference between the two predator treatments divided by the maximum respiration at each sample period) for each soil (Circle = Intact no-till soil, Triangle = Crushed no-till soil, Square = Tilled soil) and temperature (panel A = 15 °C, panel B = 25 °C). The proportion of respiration attributable to predation was higher in no-till soils than tilled soil and was influenced by crushing, but only at the higher temperature. Error bars represent propagated 95% confidence intervals about the mean of cumulative respiration.

are consistent with others' (Clarholm, 1981; Frey et al., 2001; Murase et al., 2006; Rønn et al., 2012) and offer further support for the applicability of the "microbial loop" concept to nutrient mineralization in soil systems (Adl and Gupta, 2006). The magnitude of the predation-induced increase in respiration depended on the temperature and soil structure treatment (Fig. 2). At the higher temperature, an effect of aggregate structure became apparent, with predation contributing to a greater increase in respiration in crushed soil than in intact aggregates ($P < 0.01$; General linear model ANOVA). This same temperature-dependent effect of crushing was also seen in the natural community controls, where respiration was significantly increased by crushing ($P = 0.002$; General linear model ANOVA) at the higher, but not the lower temperature. The lack of a consistent increase in respiration upon aggregate crushing (Fig. 1A) suggests that this level of disaggregation did not make substrate more accessible to decomposers, which is inconsistent with previous studies (Tisdall and Oades, 1982; Golchin et al., 1997). This discrepancy might be explained by the weaker role of transient organic binding agents (e.g., plant detritus and fungal hyphae) and the much stronger role of organic matter bound to metals and short-range-order minerals in the macroaggregate formation of Andisols compared to non-volcanic soils (Asano and Wagai, 2014).

Net nitrogen transformations displayed a discrepancy between natural and artificial communities. After incubation, both artificial communities showed a net loss of NO_3–N (this was expected due to the absence of any nitrifying taxa) while the natural controls showed significant gains, except for in tilled soils. Natural controls showed a substantial net decrease in NH_4–N while both artificial communities displayed a net increase. In artificial communities, the presence of predators resulted in significantly higher net ammonification of N ($P < 0.0005$, One-way ANOVA; Fig. 3). These results are consistent with previous work (Woods et al., 1982; Frey et al., 1985; Weekers et al., 1993) which demonstrated the ability of

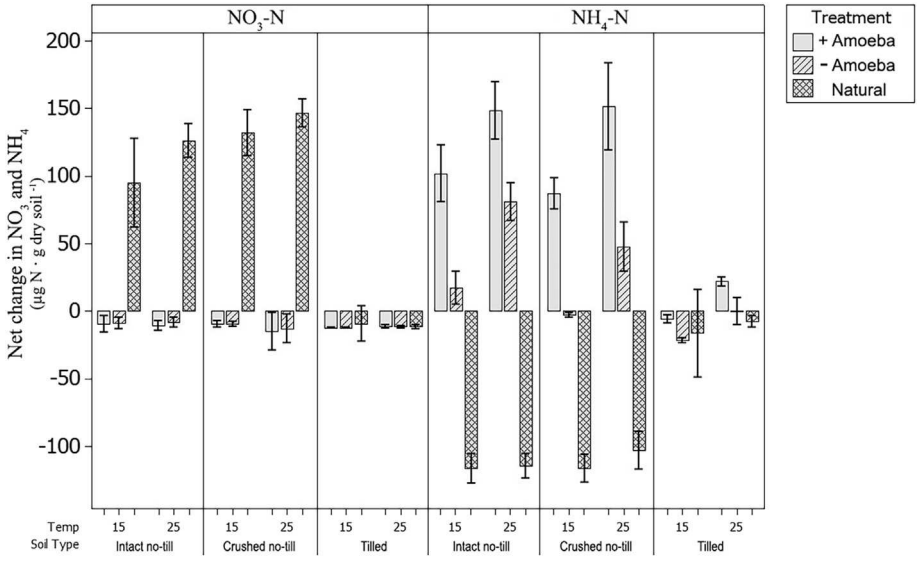

Fig. 3. Net nitrification and ammonification. The net change (μg N · g dry soil^{-1}) in NO_3–N (left panel) and NH_4–N (right panel) for each treatment after incubation. Soil treatments are listed below incubation temperature at the bottom of the figure; Solid bars = artificial community + predators (amoebae), Diagonally hatched bars = artificial community with no predators (bacteria only), Crosshatched bars = natural community controls; Temperature in °C.

amoebae to stimulate N mineralization by stimulating the turnover of bacterial biomass, but this study is the first to show that the magnitude of this effect (the predator-induced proportionate increase in N mineralization) is influenced by soil structure and temperature. The disaggregation effect on predation-induced mineralization in the no-till soils was significantly greater under the higher temperature for C ($P < 0.01$; Fig. 2) and marginally greater for N ($P = 0.06$; Fig. 3) mineralization, possibly suggesting the coupling of C and N mineralization.

At least two processes could account for the interactive effect of temperature and structure on predation-induced nutrient dynamics. One is that higher temperatures favored bacterial growth, but that the ability of amoebal predators to take advantage of more abundant prey was limited in soils with intact aggregate structure due to reduced accessibility. However, the previous incubations of bulk soils from the same two sites showed little change in microbial biomass C pool with increasing temperature from 15 to 35 °C (Wagai et al., 2013). Another is that predation efficiency could have been driven by temperature-induced shifts in predator species with differing trophic niches.

Previous work with amoebae in soils has suggested that their morphology may make them less affected by changes to physical structure (Elliott et al., 1980) due to their unique trophic mode which allows them to access bacterial prey unavailable to other predators due to size-restricted pore space associated with soil texture and aggregate structure (Young and Ritz, 2000). However, consistent with the natural controls (which contained abundant amoeboid predators), the predation-induced surges in respiration in our artificial community appeared to be limited by intact aggregate structure. It has previously been demonstrated that soil amoeba populations quickly respond to increasing bacterial populations and also that initial population densities of the predators and prey shape amoebal responses to increased bacteria (Kuserk, 1980). Further, amoebae are less responsive to temperature changes than are bacteria (García et al., 2010). Of the three predator species used in this study, only *A. polyphaga* and *D. discoideum* were recoverable from microcosms after incubation. Of the recoverable species, *A. polyphaga* differs from *D. discoideum* in that the former has a higher ideal temperature range and finer feeding pseudopodia (Marciano-Cabral and Cabral, 2003). The finding that amoebal predation efficiency was only increased by destroying aggregate structure in the warmer soils (along with congruent results from natural controls) suggests that aggregate structure may provide a sufficient refuge from amoebal predation for bacterial populations, but that this may only limit the influence of amoebal predation on C turnover once prey populations have reached a threshold density. More precise quantification of both predators and prey throughout incubations would be needed to test this hypothesis.

Soil C and N dynamics in the field are commonly affected by aggregate disruption (e.g., slaking and tillage), temperature fluctuation (e.g., diurnal, seasonal) as well as soil food web structure. While the potential role of soil food web on C and N dynamics and its temperature sensitivity has been studied in a few cases (Berg et al., 2001; Lueders et al., 2006; Whiteley et al., 2006; Bell et al., 2010), our study appears to be the first to show the interactive effect of soil aggregate structure and temperature on the predation-induced increase in C and N mineralization and suggests the importance of accounting for these interactions when predicting soil C and N dynamics under climate change scenarios, particularly in environments that are predicted to experience significant warming. Future work should assess whether the effect found here for Andisols is applicable to other soil types and test the underlying mechanisms behind the observed pattern.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2015.11.021.

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