

Trophic level mediates soil microbial community composition and function

Jane M. Lucas^{a,1}, Steven G. McBride^{b,1}, Michael S. Strickland^{a,*}

^a Department of Soil and Water Systems, University of Idaho, Moscow, ID, 83844, USA

^b Department of Biological Sciences, Virginia Tech, Blacksburg, VA, 24061, USA

ARTICLE INFO

Keywords:

Bacteria
Fungi
Soil carbon
Collembola
Pseudoscorpion
Extracellular enzyme activity

ABSTRACT

Soil microbial communities drive ecosystem processes, and technological advances have led to an unprecedented understanding of these communities. Yet microbes are only one constituent of soil communities. Understanding how soil microbes will respond to changes in the trophic levels of soil food webs, particularly in combination with inputs of labile carbon resources, is vital for a complete picture of belowground dynamics. Here we manipulate the trophic levels of soil communities, creating a microbe treatment, a microbivore treatment, and two predator treatments that test between consumptive and non-consumptive effects. We then exposed these communities to glucose additions that simulate either the rhizosphere or bulk soil. We found that trophic levels, with and without glucose addition, lead to shifts in microbial community composition and function. Specifically, we observed that the presence of increasing trophic levels led to distinct bacterial communities compared to treatments containing only microbes, and the presence of the predator led to the most distinct shifts compared to the microbe treatment. Not surprisingly, soil respiration was greater in the rhizosphere compared to the bulk soil with the microbe treatment exhibiting greater and lesser respiration compared to the other treatments in the rhizosphere versus the bulk soil, respectively. However, the similarity in respiration between treatments was driven by different underlying processes where the presence of the predator leads to increased microbial biomass and microbial efficiency. In fact, trophic levels, compared to the availability of labile carbon, had a greater influence on microbial efficiency. This suggests that trophic levels of soil communities should be considered when attempting to understand the effect of soil microbial communities on ecosystem processes.

1. Introduction

Microbial communities are the preeminent players in soil ecosystems. Not surprisingly, these diverse communities influence processes from litter decomposition (Strickland et al., 2009; Glassman et al., 2018), to nutrient cycling (Fierer et al., 2013), to soil organic C stabilization (Grandy and Neff, 2008; Bradford et al., 2013; Cotrufo et al., 2013). In fact, the importance of soil microbial communities to varied ecosystem processes has catalyzed an increased effort to include the complexity of these communities in ecosystem models, often leading to increased accuracy in parameter estimates (Wieder et al., 2014; Grandy et al., 2016). Yet this focus on microbial communities often misses the complexity associated with the species interactions that perpetuate throughout the soil food web (Grandy et al., 2016; Soong and Nielsen, 2016).

In part, this disregard for food web interactions may be due to the expectation that soil food webs are largely constrained by resource

availability (i.e., bottom-up controls) of plant derived inputs (Thakur, 2019). In particular, focus on labile C sources (e.g., litter leachates and root exudates) illustrates that these resources fuel the bulk of microbial activity in soil and account for up to 30% of soil respiration (van Hees et al., 2005). But these labile inputs have often been overlooked from the context of the soil food web (Bradford, 2016). Our understanding of labile C inputs, which typically enter soils as root exudates or litter leachates, suggests that they are responsible for soil organic matter formation in part because of the increased efficiency by which soil microbial communities can process these inputs, leading potentially to increased incorporation of microbial products on mineral surfaces (Cotrufo et al., 2013; Lehmann and Kleber, 2015). In other words, the greater the microbial C-use efficiency, the greater the production of microbial residues, and in turn the greater the potential for soil organic C formation. While recent research highlights the likelihood of this pathway, much of its theoretical underpinnings are microbial-centric and fail to consider the role of soil food webs, and in particular

* Corresponding author. University of Idaho, Department of Soil and Water Systems, 875 Perimeter Drive MS 2340, Moscow, ID 83844-2340, USA.

E-mail address: mstrickland@uidaho.edu (M.S. Strickland).

¹ J.M.L. and S.G.M. contributed equally to this work.

trophic levels and subsequent top-down controls.

Classic research on soil food webs has illustrated the potential for carbon and nutrient cycling to be influenced by food web structure. For instance, the seminal work of Hunt et al. (1987) illustrated the importance of energy channels and the role key groups (i.e., amoebae and bacterivorous nematodes) play in nitrogen (N) mineralization dynamics. However, the integration of soil food webs with our current understanding of soil processes has recently lagged. This lag was highlighted by Bradford (2016), who stressed that species interactions have not been incorporated into soil food web theory, creating limitations in our understandings of soil systems. Of these interactions, trophic interactions have often proved fundamental to our understanding of aboveground communities but may be equally as important to our understanding of belowground communities (Hawlena and Zaguri, 2016).

When considering aboveground top-down controls, research shows that predators can affect prey via both consumptive and non-consumptive effects (Hawlena and Schmitz, 2010a; Guariento et al., 2015). While consumptive effects are widely studied in aboveground and aquatic systems, their role in soil systems is more sporadic. One example of belowground consumptive effects highlighted the top-down regulatory role that predatory nematodes play in regulating bacterial but not fungal biomass (Wardle and Yeates, 1993). Another example found top-down and bottom-up interactions as predator abundance was correlated with increased retention of C from a simulated root exudate (Strickland et al., 2012). It is likely that these observed predatory effects are due to consumptive behaviors but there is also the possibility that non-consumptive effects play a role.

Like consumptive effects, non-consumptive effects in aboveground ecosystems are known to regulate prey populations, and influence ecosystem processes. For instance, non-consumptive effects exert top-down control over litter decomposition rates by inducing changes in prey physiology (Hawlena et al., 2012), and at the ecosystem-scale these effects can lead to greater C retention in plant biomass and decreased soil respiration (Strickland et al., 2013). In belowground systems non-consumptive predator cues lead to decreased soil respiration and nitrogen content (Zhao et al., 2013; Sitvarin and Rypstra, 2014). Such results highlight the potential for trophic levels, in general, and predator effects, specifically, to influence soil processes. However, much of this research has focused on how these top-down controls affect litter decay or surface detritus, overlooking the interaction between trophic levels and labile inputs of C, inputs similar to those observed in the rhizosphere.

Here we examine the effect of trophic level on microbial efficiency, and microbial community composition and function in microcosms that simulate either the rhizosphere (i.e., received labile C additions) or bulk soil (i.e., did not receive labile C additions). For this experiment we manipulated trophic level creating a microbe only treatment, a microbivore treatment, and two predator treatments that enabled us to test between consumptive and non-consumptive (i.e., fear) effects. We define fear to represent the potential behavioral changes that will occur in collembola in order to avoid predator encounters. We predict the classic theory of top-down versus bottom up controls (Moore et al., 2003) will be maintained, where the overall effect of trophic level will be mediated by whether the soil simulates a rhizosphere or not. Specifically, we expect that microbial communities are carbon limited and will therefore respond strongly to the addition of labile C. However, when C-limitation is removed we also predict an effect of trophic level on composition. With regards to microbial function, we predict that microbial efficiency, as determined by mass-specific respiration, will be greatest in the simulated rhizosphere compared to the bulk soil (Sokol et al., 2019) but that this efficiency will be mediated by trophic level. In the simulated rhizosphere, we predict that the presence of the microbivore will lower microbial biomass compared to when the predator is present due to the lack of control on microbivore grazing when predators are absent. Furthermore, if non-consumptive effects play a dominant role then the effect on microbial efficiency will be similar to the

predator treatment. Ultimately, we expect that microbial efficiency is not simply a product of bottom-up controls but is affected by top-down controls as well.

2. Methods

2.1. Experimental design and microcosm respiration

To examine the potential for soil trophic level, with and without C limitation, to effect microbial efficiency, community composition, and function, we employed a microcosm approach. Soil (0–10 cm depth) for use in the microcosms was sourced from a mixed deciduous forest located at Kentland Farm, VA, USA (N 37.1977, W –80.5818). Soils at this location are Ultisols classed as fine, mixed, semiactive, mesic Typic Hapludults of the Unison and Braddock series (Soil Survey Staff; accessed on July 11, 2019). Prior to addition, microarthropods were extracted from the soil via Berlese funnels. Arthropods were extracted live by fitting each funnel with a container containing ~2 cm of cured and dried plaster of Paris. Water was added to each of these containers to create a moist environment which reduced mortality of extracted arthropods. From these extracted arthropods, we collected collembola (*Folsomia candida*) and pseudoscorpions (*Microbisium* sp.) using a modified insect aspirator. Species were identified using a stereomicroscope after first preserving representative individuals in 90% ethanol. Identification was done using the keys from Dindal (1990), Janssens and Lebeaux's pictorial key (<http://www.collembola.org/key/fkfr.htm>), and Buddle (2010). The collembola sourced from the soil were used to start colonies for stocking soil microcosms following the procedures outlined in Moore et al. (2000). Pseudoscorpions were kept in isolated containers until the start of the experiment. Additional pseudoscorpions were collected via live Berlese funnel extractions to ensure sufficient numbers for each microcosm.

Microcosms were constructed by first homogenizing the soil from which arthropods had been extracted and then adding 50 g of dry weight equivalent soil to glass jars (~473 mL). Soil was adjusted to 65% water-holding capacity, which is optimal for microbial activity, and allowed to equilibrate for 2 weeks prior to implementing experimental treatments. In total 40 microcosms were constructed (4 trophic level treatments × 2 glucose additions × 5 replicates). To manipulate trophic level, we either added no microarthropods to the soil, collembola (8 collembola added), collembola and an encaged pseudoscorpion, or collembola and a free-roaming pseudoscorpion. The pseudoscorpion was caged by placing it in an 8 mL vial, containing a thin coating of plaster of Paris to maintain moisture. The vial was capped with 53 µm mesh and placed on its side on the soil surface. This set up enabled us to create 4 treatments aimed at assessing trophic level: a 'microbe' only treatment (i.e., soil with no microarthropods), a microbivore only treatment (i.e., soil with the addition of only collembola), an indirect predation treatment (i.e., soil with the addition of collembola and an encaged pseudoscorpion), and a direct + indirect predation treatment (i.e., soil with the addition of collembola and a free-roaming pseudoscorpion), referred to from here on as microbe, collembola, fear, and predator treatment, respectively. To half of the microcosms, we added glucose weekly at a rate of 260 µg C g dry wt soil⁻¹ day⁻¹. This rate was aimed to simulate high C availability associated with the soil rhizosphere (Strickland et al., 2015). The other half of the microcosms received no addition of glucose. Together these additions created a high and low resource environment and allowed us to test trophic level in the presence and absence of bottom-up C-limitation on microbial community composition, enzyme activity, and microbial efficiency.

After microcosms were constructed, we monitored respiration for 117 days using an infrared gas analyzer (IRGA; Model LI-7000, Li-Cor Biosciences, Lincoln, Nebraska, USA). Each microcosm was fitted with a lid, complete with input and output ports. Microcosms were then connected to a multiplexer (Model LI-8150, Li-Cor Biosciences, Lincoln, Nebraska, USA) coupled to the IRGA. Respiration in each microcosm

was determined over ~2 min, and all microcosms were measured 16 times (i.e. sampling day 1, 5, 12, 19, 26, 33, 40, 47, 61, 68, 75, 82, 89, 103, 110, and 117) across the 117-day experiment. We then calculated cumulative respiration for this period via integration.

2.2. Determination of soil properties and microbial efficiency

After the 117-day experiment, we destructively harvested soil microcosms in order to determine the effect of substrate addition and trophic level on soil pH, active microbial biomass, mineralizable soil C, particulate organic matter (POM) C and N, and mineral-associated soil C and N. Soil pH (1:1, soil:water by volume) was determined on a benchtop pH meter. Active microbial biomass was determined via substrate induced respiration (SIR) following Fierer et al. (2003). Briefly, SIR was determined using soil slurries (4 g dry mass equivalent soil) that were pre-incubated for 1 h with excess autolyzed yeast substrate while shaking, followed by a 4-h static incubation at 20 °C. After incubation, SIR was determined on an IRGA. We converted the SIR rate to equivalents of microbial biomass C using the equation described in Phillips et al. (2011).

Mineralizable C, an estimate of bioavailable C (Fierer et al., 2007), was determined via 22-d C-mineralization assays. Soils were maintained at 20 °C and 65% water-holding capacity with periodic determination of respiration using the same static incubation technique described for SIR, except soils were incubated for 24 h prior to measuring headspace CO₂ concentrations. Total mineralizable C was estimated by integrating CO₂ production across time.

We used a fractionation method to differentiate between the faster cycling particulate organic matter (POM) and slower cycling mineral-associated soil C and N pools (Schlesinger and Lichter, 2001), using the method described in Bradford et al. (2008b). Air-dried soil (10 g) from each microcosm was dispersed with sodium hexametaphosphate via shaking (18 h), and then passed through a 53-μm sieve. Mineral-associated material was considered <53 μm, and POM material was >53 μm. Both soil fractions were dried (105 °C) and ball-milled to a fine powder. Percentage C and N were determined using a NA1500 CHN analyzer (Carlo Erba, Milan, Italy).

To estimate microbial efficiency, we calculated microbial mass-specific respiration using a procedure similar to Wepking et al. (2017) and Bradford et al. (2008a). Briefly, we used the initial measurement of our C-mineralization assay, which corresponds to soil respiration after 117 days of exposure to substrate and trophic treatments, divided by our estimate of active microbial biomass (i.e., SIR). We expect that greater respiration per unit microbial biomass compared to lower respiration per unit microbial biomass is indicative of decreased microbial efficiency (Wepking et al., 2017; McBride and Strickland, 2019).

2.3. Determination of microbial community composition and function

To assess microbial community composition, we determined both bacterial and fungal communities via marker gene sequencing. DNA was extracted from each soil sample using the MoBio® PowerSoil kit (MoBio Laboratories, Inc., Carlsbad, CA, USA), according to the manufacturer's protocols. Ribosomal marker genes were amplified using 2-step PCR in accordance with the Earth Microbiome Project protocol for 16S and ITS sequencing (www.earthmicrobiome.org). We used the ITS1/ITS2 and the 515F/806R primer pairs for fungi and bacteria, respectively. After the first round of PCR, sequences were cleaned using ExoSAP-IT™ PCR clean-up reagent (Affymetrix Inc., Santa Clara, CA, USA), according to the manufacturer's protocol. During the second round of PCR, unique barcoded primers were added to each sample. After the second round of PCR, we cleaned and normalized samples using SequelPrep™ 96-well plates (Invitrogen, Carlsbad, CA, USA). We pooled equimolar amounts of DNA, and sequenced our amplicon pools on an Illumina MiSeq instrument using 2 × 300 bp sequencing kits at the Genomics Resource Core (GRC) sequencing facility at the University of Idaho. Controls were

used throughout the laboratory process to ensure there were no contaminants.

Raw sequences were first demultiplexed by the University of Idaho's GRC using the program dbcAmplicons (Uribe-Convers, 2016). This process also removed barcodes and primers from sequences. Paired sequences were then processed using the DADA2 pipeline (Callahan et al., 2016), which is designed to resolve exact biological sequences from Illumina sequence data and does not involve sequence clustering (Leff, 2016). Paired sequences were trimmed to uniform lengths, dereplicated, and the unique sequence pairs were denoised using the 'dada' function, accounting for errors through the model generated with the 'learnErrors' command. We then merged paired-end sequences and removed chimeras. Taxonomy assignments were determined using the Silva (ver. 132, Quast et al., 2013) and the UNITE dynamic general release (ver 01.12.2017, Abarenkov et al., 2010) databases for bacteria and fungi, respectively. To account for differences in sequencing depths, we rarefied samples to 3626 and 8668 sequences per sample for fungi and bacteria, respectively.

To assess microbial community function, we measured extracellular enzyme activity.

Following the procedure outlined by DeForest (2009), we determined activity of the following hydrolytic enzymes: cellobiohydrolase (CBH; involved in cellulose degradation), acid phosphatase (AP; hydrolyzes phosphomonoesters), N-acetyl-β-glucosaminidase (NAG; involved in chitin degradation), and β-glucosidase (BG; hydrolyze cellulose, releasing glucose) as fluorescence of the methylumbelliferyl (MUB)-linked substrates β-D-cellobioside, phosphate, N-acetyl-β-D-glucosaminide, and β-D-glucopyranoside. After homogenizing ~1 g of dry weight soil in 100 mL of sodium acetate buffer (pH adjusted to microcosm soil pH), the resultant soil slurry was combined with 50 μL of substrate in a 96-well microplate. After incubation, fluorescence was measured at excitation wavelength of 360 nm and an emission wavelength of 450 nm on a fluorometric plate reader (Model Infinite M200; Tecan Group Ltd, Männedorf, Switzerland). Enzyme activity is expressed as micromoles of substrate converted (μmol h⁻¹ g dry wt soil⁻¹).

2.4. Statistical analyses

The effect of substrate addition, trophic level, and their interaction on cumulative microcosm respiration, soil pH, active microbial biomass, soil C and N pools, and microbial efficiency were investigated using analysis of variance (ANOVA). Post-hoc analysis to determine pairwise differences was conducted using TukeyHSD. When necessary, data were log-transformed to meet assumptions of normality. Microbial community composition, and extracellular enzyme activity were analyzed using permutational MANOVA (perMANOVA) and visualized using principal coordinates analysis (PCoA). Pairwise comparisons between treatments were also analyzed via perMANOVA and we tested for homogeneity of dispersions from the centroids via betadisper tests (Anderson et al., 2008). For bacterial and fungal communities (at the ASV level and after square-root transformation) Bray-Curtis distances were analyzed, and for extracellular enzyme activity Euclidean distances were analyzed. ANOVA was conducted in R (R Core Team, 2012), and microbial community and extracellular enzyme activity analyses were conducted in Primer (Clarke and Gorley, 2006). Significant treatment effects were considered at $P < 0.05$, and marginal significance was considered at $P < 0.10$. Additional results showing respiration dynamics, interaction plot for mass specific respiration, bacterial community taxa, and specific extracellular enzymes can be found in the Supplementary Material.

3. Results

3.1. Effect of trophic level on microcosm respiration, microbial efficiency, and soil properties

Microcosm respiration across the 117-day experiment (Fig. 1A), was

significantly affected by glucose addition ($F_{1,32} = 8803.9$; $P < 0.001$). The addition of glucose lead to an 8.4-fold increase versus all treatments that received no glucose. While no main effect of trophic level was detected ($F_{3,32} = 0.46$; $P = 0.71$), a significant interaction between trophic level and substrate addition ($F_{3,32} = 18.64$; $P < 0.001$) was observed. This interaction is due to greater cumulative microcosm respiration for the microbe treatment compared to the other trophic level treatments without glucose, versus lower cumulative respiration for the microbe treatment compared to the other trophic level treatments when glucose was added (Fig. 1A).

For active microbial biomass (Fig. 1B), no interaction between glucose addition and trophic level was observed ($F_{3,32} = 0.68$; $P = 0.57$), but main effects of both glucose addition ($F_{1,32} = 1101.34$; $P < 0.001$) and trophic level ($F_{3,32} = 5.20$; $P < 0.01$) were observed. Not surprisingly, the addition of glucose led to a 7.9-fold increase in active microbial biomass compared to microcosms that did not receive glucose. More surprising for trophic level was the observed 1.35 and 1.37-fold increase in active microbial biomass regardless of glucose additions

for the predator treatment compared to the microbe and collembola treatments, respectively (Fig. 1B). Active microbial biomass for the fear treatment was intermediate (Fig. 1B).

Compared to active microbial biomass, we observed the opposite trend of trophic level on microbial efficiency (i.e. mass specific respiration; Fig. 1C). That is the predator treatment was associated with the greatest microbial efficiency (or lowest mass specific respiration), the microbe and collembola treatments had the lowest, and the fear treatment was intermediate ($F_{3,32} = 8.40$; $P < 0.001$). Surprisingly, glucose addition did not elicit a significant effect ($F_{1,32} = 0.02$; $P = 0.89$), albeit a marginally significant interaction with trophic level was observed ($F_{3,32} = 2.71$; $P = 0.06$). This interaction is likely due to a decrease in microbial efficiency observed for the microbe treatment when glucose was added compared to when no glucose was added. However, the other trophic treatments tended to shift less dramatically between the two glucose treatments.

For most of the remaining soil properties, trophic level had little to no effect, but glucose addition tended to exhibit a significant effect

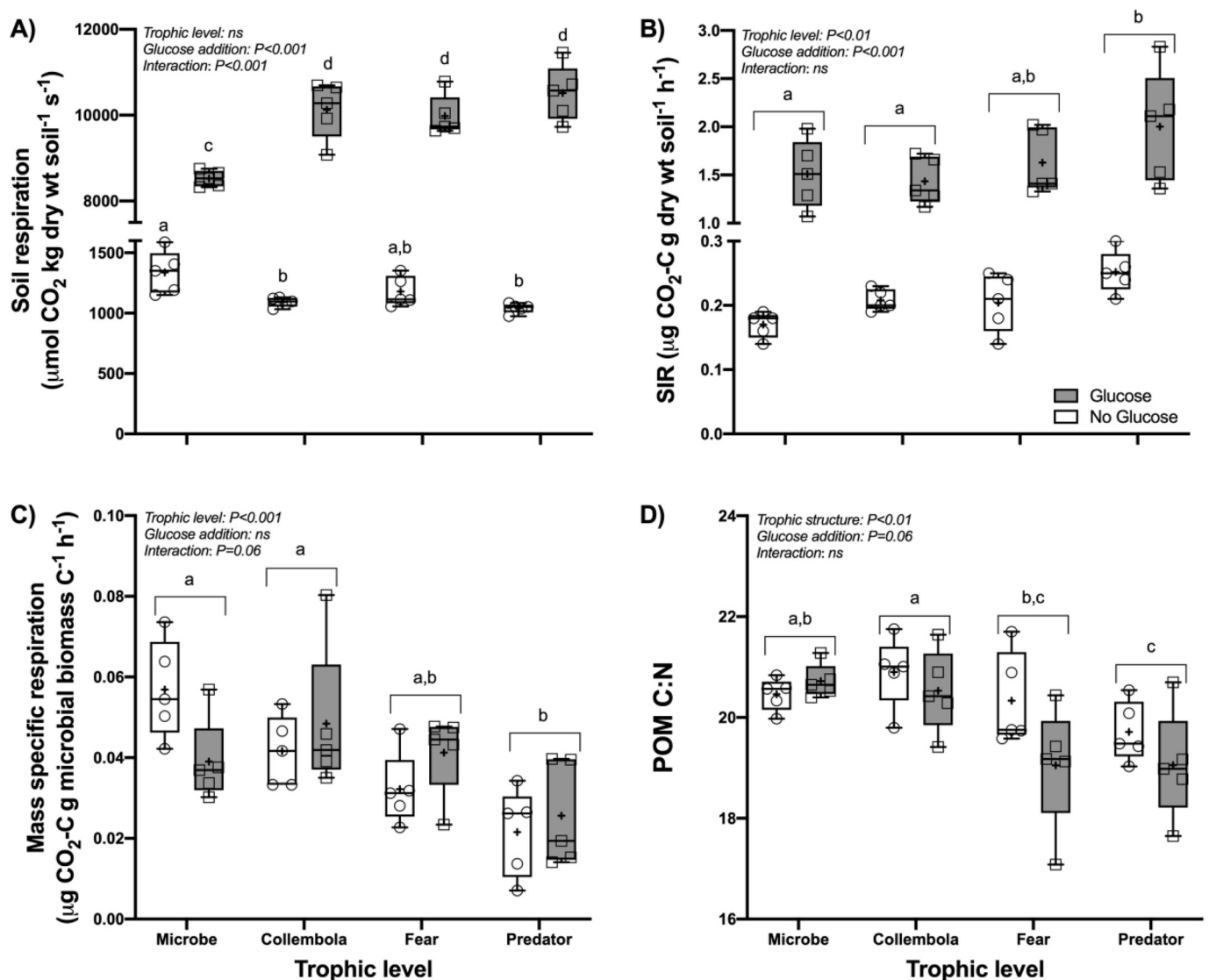


Fig. 1. The response of microcosm respiration (A), active microbial biomass as determined by substrate induced respiration (B), microbial mass specific respiration (C), and particulate organic matter (POM) C:N (D) to glucose additions (no glucose vs glucose) and trophic level (microbe, collembola, fear, and predator). For all 4 panels, boxplots show the median, 25th and 75th percentiles, and minimum and maximum values, means are denoted with '+'. All data points are represented, with trophic level treatments identified in the legend in panel A. Glucose additions are denoted by grey bars and square symbols; no glucose treatments are denoted by white bars and circle symbols. Note when a significant interaction is observed, pairwise comparisons for all treatment combinations are shown (i.e. panel 1A). When a significant trophic level effect is observed, pairwise comparisons bracket both the glucose and no glucose additions for that treatment.

(Table 1). This general substrate effect was primarily due to increases in soil C pools and soil pH with glucose addition (Table 1). A key exception to this general trend, was the C:N ratio of POM material which was significantly affected by trophic level ($F_{3,32} = 6.56$; $P < 0.01$). The POM C:N ratio associated with the predator treatment was lower than that associated with either the microbe or collembola treatment, with the fear treatment again intermediate (Fig. 1D). Additionally for POM C:N, glucose addition was marginally significant ($F_{1,32} = 3.96$; $P = 0.06$), and no interaction was noted ($F_{3,32} = 1.56$; $P = 0.22$).

3.2. Trophic level and C availability shift microbial community composition and function

For bacterial community composition (Fig. 2), we observed interaction between glucose addition and trophic level (pseudo- $F_{3,28} = 1.25$; $P < 0.05$). Centroid dispersions did not differ between trophic treatments ($F_{3,32} = 0.66$; $P = 0.55$) for bacterial communities, but a marginal difference was observed for substrate addition ($F_{1,34} = 3.74$; $P = 0.06$). This marginal difference was primarily due to greater dispersion associated with the addition of glucose compared to the control. We investigated the interaction between trophic level and glucose addition, by examining the effect of trophic level on bacterial community structure either with or without glucose addition (Fig. 2). Without glucose addition (pseudo- $F_{3,18} = 1.14$; $P < 0.05$), the predator treatment differed significantly from the microbe treatment, with the collembola and fear treatments intermediate (Fig. 2A). With glucose addition (Fig. 2B; pseudo- $F_{3,16} = 1.27$; $P < 0.05$), both the fear and predator treatment differed from the microbe treatment, with the collembola treatment intermediate (Fig. 2B). Potential drivers of these differences in community composition could be attributed to multiple bacterial families. Additionally, we observed significant effects of both glucose addition ($F_{1,28} = 36.78$; $P < 0.001$), and trophic level ($F_{3,28} = 4.72$; $P < 0.01$), but no interaction ($F_{3,28} = 2.29$; $P = 0.10$), on bacterial richness, with greater richness observed for the microbe treatment compared to both the collembola and predator treatments, and greater richness associated with the addition of glucose.

For fungal community composition (Fig. 3), we observed a significant main effect of glucose addition (pseudo- $F_{1,28} = 36.78$; $P < 0.001$), a marginally significant effect of trophic level (pseudo- $F_{3,28} = 1.19$; $P = 0.06$), but no significant interaction between trophic level and glucose addition (pseudo- $F_{3,28} = 1.10$; $P = 0.16$). Centroid dispersions did not differ for trophic level ($F_{3,32} = 1.47$; $P = 0.29$) but did for glucose addition ($F_{1,34} = 25.38$; $P < 0.001$). Like the bacterial community, dispersion for fungal communities was greater with the addition of glucose (Fig. 3A). The marginally significant effect observed for trophic level was due to differences in fungal community composition between the collembola and microbe treatments (Fig. 3A). This is potentially driven by a marginally significant effect of trophic level associated with class Agaricomycetes within the phylum Basidiomycota ($F_{3,28} = 2.75$; $P = 0.06$), where on average the collembola treatment had lower abundance than the other three treatments (Fig. 3B). Additionally, we observed a significant effect of glucose addition ($F_{1,28} = 28.73$; $P < 0.001$), but neither trophic level ($F_{3,28} = 0.32$; $P = 0.81$) nor an interaction ($F_{3,28} = 0.90$; $P = 0.45$), on fungal richness. Fungal richness declined with the addition of glucose.

For microbial community function (Fig. 4), determined via profiles of extracellular enzyme activity, we observed an interaction between glucose addition and trophic level (pseudo- $F_{3,30} = 6.16$; $P < 0.01$). Centroid dispersions did not differ between trophic treatments ($F_{3,34} = 3.49$; $P = 0.06$), but did for glucose addition ($F_{1,36} = 16.14$; $P < 0.01$). We further investigated the interaction between trophic level and glucose addition, by examining the effect of trophic level on enzyme activity either with or without glucose addition (Fig. 4). Without glucose addition (pseudo- $F_{3,19} = 2.26$; $P < 0.05$), enzyme profiles associated with the collembola treatment differed from both the fear and predator treatments, the microbe treatment was intermediate (Fig. 4A). With

Table 1
Soil C and N pools, and soil pH after 117 days of exposure to glucose addition (i.e. no glucose vs glucose) and trophic level treatments (i.e. microbes, collembola, fear, and predator). Shown below variable are the statistical results for the main effects (glucose addition and trophic treatment) and interaction (glucose addition $\times$ trophic treatment) for that specific variable. Significant P-values are indicated in bold while the actual P-value is shown for marginally (i.e. $P < 0.10$) significant results.

Glucose addition	Trophic Level	POM C (mg g dry wt soil ⁻¹)	Mineral C (mg g dry wt soil ⁻¹)	Total C (mg g dry wt soil ⁻¹)	POM N (mg g dry wt soil ⁻¹)	Mineral N (mg g dry wt soil ⁻¹)	Total N (mg g dry wt soil ⁻¹)	POM C:N	Mineral C:N	Total C:N	Mineralizable C (μg g dry wt soil ⁻¹)	Soil pH
No Glucose	Microbes	19.95 ± 1.02	16.50 ± 0.79	36.45 ± 0.80	0.98 ± 0.05	1.44 ± 0.06	2.41 ± 0.06	20.46 ± 0.15	11.49 ± 0.11	15.13 ± 0.19	122.60 ± 6.20	4.13 ± 0.02
	Collembola	21.49 ± 0.93	15.54 ± 0.91	37.03 ± 1.49	1.03 ± 0.04	1.35 ± 0.09	2.38 ± 0.08	20.90 ± 0.31	11.56 ± 0.46	15.56 ± 0.42	117.00 ± 12.0	4.15 ± 0.06
	Fear	21.52 ± 0.77	16.49 ± 0.55	38.01 ± 0.85	1.06 ± 0.02	1.47 ± 0.06	2.53 ± 0.07	20.33 ± 0.41	11.28 ± 0.55	15.07 ± 0.32	107.52 ± 6.94	4.14 ± 0.04
	Predator	20.03 ± 0.61	14.39 ± 0.99	34.42 ± 0.95	1.02 ± 0.02	1.10 ± 0.20	2.11 ± 0.20	19.71 ± 0.27	14.66 ± 2.15	16.79 ± 1.38	103.75 ± 9.06	4.17 ± 0.06
Glucose	Microbes	23.97 ± 0.80	18.25 ± 0.65	42.22 ± 0.74	1.16 ± 0.04	1.31 ± 0.05	2.47 ± 0.03	20.72 ± 0.15	13.93 ± 0.21	17.12 ± 0.22	825.61 ± 53.6	6.17 ± 0.07
	Collembola	20.99 ± 1.27	18.73 ± 1.10	39.72 ± 1.58	1.02 ± 0.05	1.41 ± 0.02	2.43 ± 0.06	20.53 ± 0.37	13.30 ± 0.83	16.33 ± 0.48	871.52 ± 61.3	5.80 ± 0.11
	Fear	21.23 ± 1.30	18.29 ± 0.74	39.52 ± 0.81	1.12 ± 0.08	1.30 ± 0.06	2.42 ± 0.05	19.05 ± 0.55	14.07 ± 0.09	16.33 ± 0.18	778.67 ± 52.5	6.01 ± 0.12
	Predator	22.43 ± 0.99	17.04 ± 1.29	39.47 ± 0.53	1.18 ± 0.07	1.24 ± 0.09	2.42 ± 0.03	19.05 ± 0.49	13.76 ± 0.10	16.30 ± 0.12	835.89 ± 136.4	5.83 ± 0.04
Trophic level:		ns	ns	ns	ns	ns	ns	$P < 0.01$	ns	ns	ns	ns
Glucose addition:		$P = 0.05$	$P < 0.01$	$P < 0.001$	$P < 0.01$	ns	ns	$P = 0.06$	$P < 0.01$	$P < 0.05$	$P < 0.001$	$P < 0.001$
Interaction:		$P = 0.08$	ns	ns	ns	ns	ns	ns	ns	ns	ns	$P = 0.06$

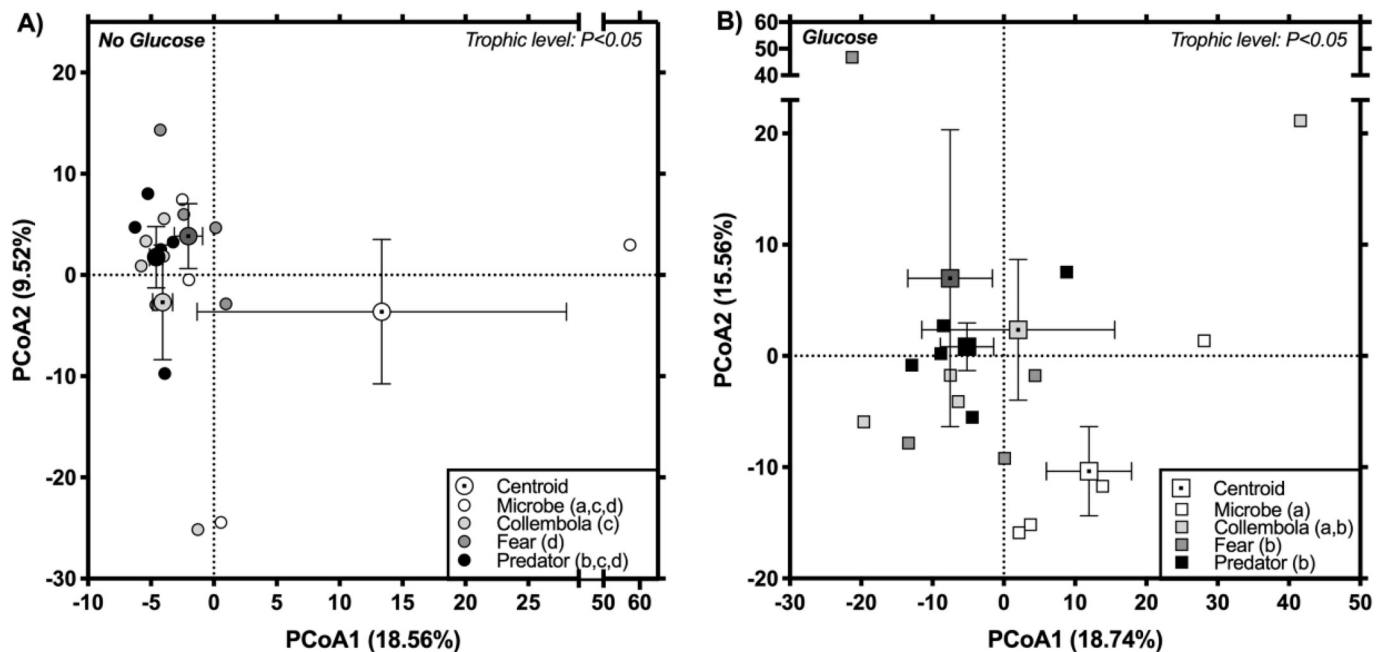


Fig. 2. Bacterial community composition associated with the four trophic levels without (A), and with (B) glucose addition. Both panels are ordination plots showing principal coordinates analysis of Bray-Curtis distances between bacterial communities. The centroid ± 1 S.E. for each trophic level is plotted as either a circle or square with central dot, for panel A and B, respectively. In the figure key (lower right-hand corner of each panel), letters denote significant pairwise differences between treatment centroids as determined via permutational MANOVA. Note that separate panels for glucose addition are shown because a significant trophic level $\times$ glucose addition interaction was observed.

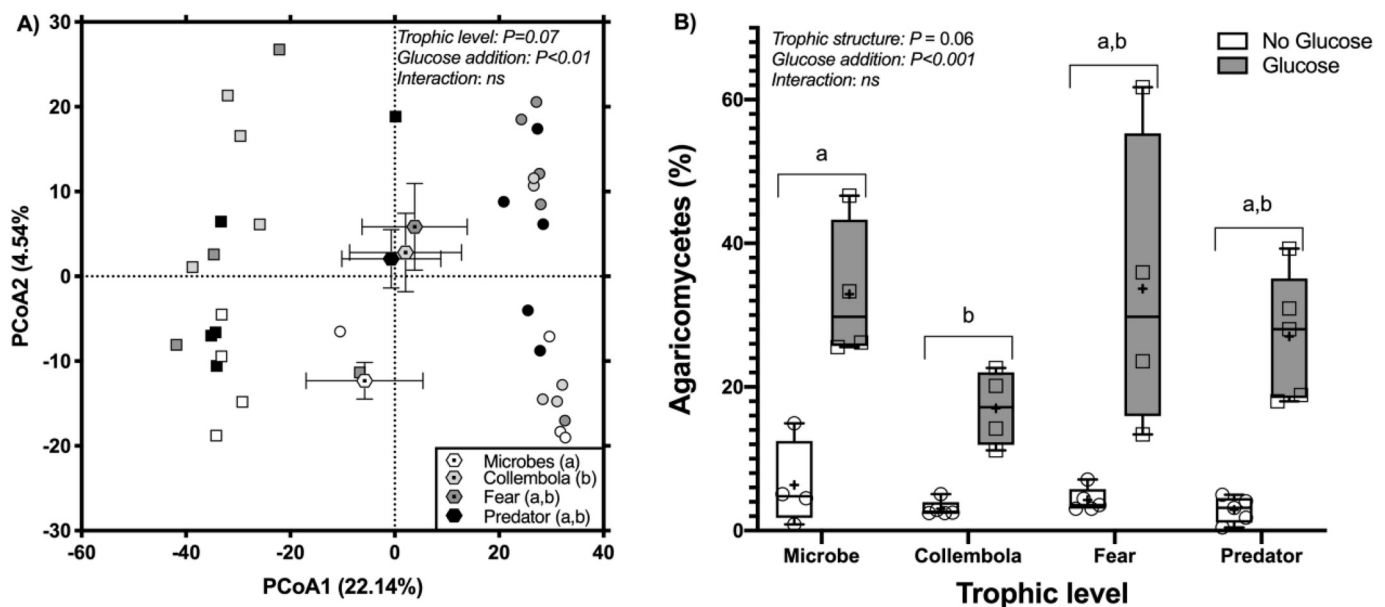


Fig. 3. Fungal community composition (A) and the relative abundance of class Agaricomycetes (B) associated with the four trophic levels across glucose additions. A) An ordination plot showing principal coordinates analysis of Bray-Curtis distances between fungal communities. The centroid ± 1 S.E. for each trophic level treatment is plotted as an octagon with central dot. In the figure key (lower right-hand corner of each panel), letters denote significant pairwise differences between treatment centroids as determined via permutational MANOVA. Squares and circles indicate microcosms that received or did not receive glucose, respectively. B) Box-plots showing the relative abundance of class Agaricomycetes (phylum Basidiomycota) for the four trophic levels. Box-plots are as described in Fig. 1. Letters denote significant pairwise differences between trophic levels as determined via Tukey's HSD test.

glucose addition (pseudo- $F_{3,17} = 5.17$; $P < 0.01$), the microbe treatment differed from the other three treatments (Fig. 4B). When examining the response of specific enzymes to trophic level and glucose addition, a significant interaction was observed for all four enzymes. In general, these interactions were due to relatively little difference in enzyme activity associated with trophic level without the addition of glucose but

with the addition of glucose, enzyme activity for the microbe treatment tended to be lower than the other trophic treatments. Although for BG and CBH, only the microbe and collembola treatments differed, with the fear and predator treatments exhibiting intermediate activities. Additionally, an interaction was observed for total enzyme activity (i.e. the sum of AP, NAG, BG, and CBH activity). The interaction associated with

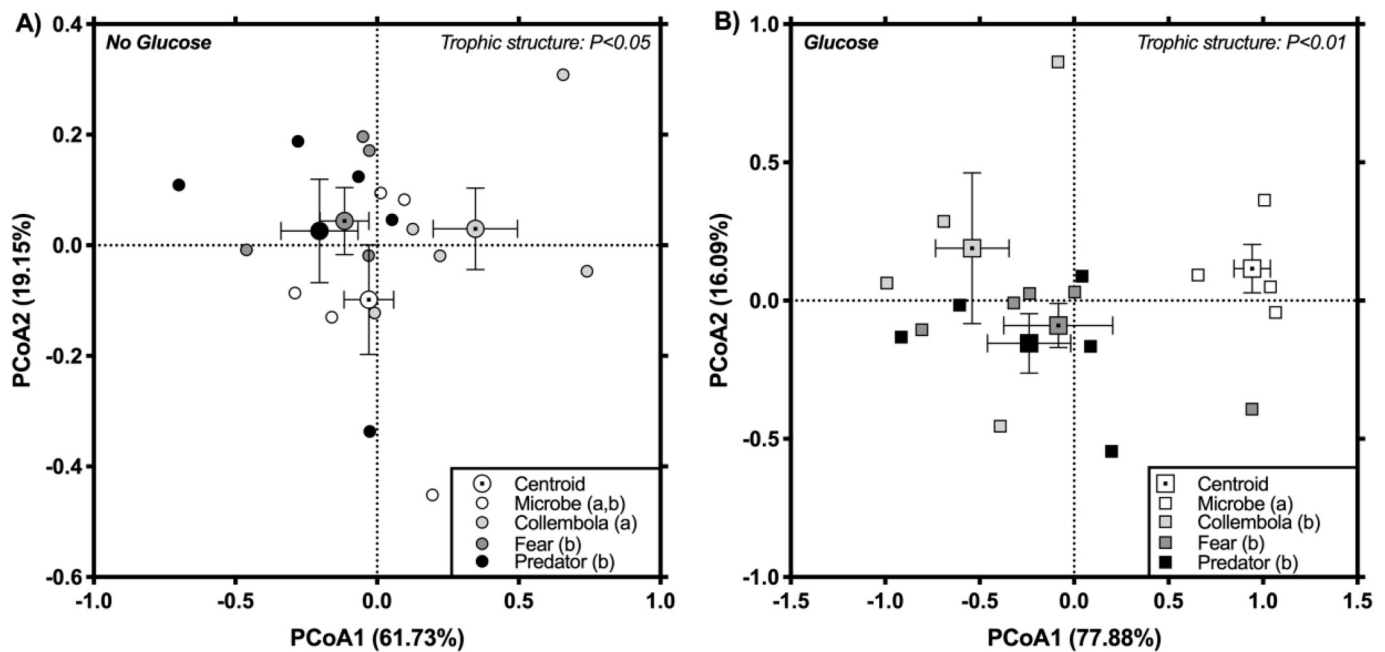


Fig. 4. Microbial community function – as determined via extracellular enzyme activity – associated with the four trophic levels without (A), and with (B) glucose addition. Both panels are ordination plots showing principal coordinates analysis of Euclidean distances between enzyme profiles. The centroid ± 1 S.E. for each trophic level is plotted as either a circle or square with central dot, for panel A and B, respectively. Trophic levels are as shown in Fig. 2. In the figure key (lower right-hand corner of each panel), letters denote significant pairwise differences between treatment centroids as determined via permutational MANOVA. Note that separate panels for glucose addition are shown because a significant trophic level $\times$ glucose addition interaction was observed.

total enzyme activity was due to no trophic level differences with glucose, but a significant difference between the collembola and predator treatments without glucose.

4. Discussion

4.1. Effect of trophic level on microcosm respiration, and microbial biomass and efficiency

In this study we sought to better understand the influence of bottom-up control and top-down controls on microbial efficiency, and microbial community composition and function. We expected that trophic level would have a stronger influence on the soil microbial community with the addition of glucose, and a weaker influence when glucose was not added. Surprisingly, though, trophic effects were observed both with and without glucose addition. While interactions between substrate addition and trophic level were noted, overall our results suggest that in this microcosm set-up trophic level (regardless of substrate limitation) can influence characteristics of the microbial community and may have ramifications for soil processes, such as C-cycling.

For cumulative microcosm respiration across 117 days, we observed an interaction between trophic level and glucose addition; the microbe only treatment had increased cumulative respiration compared to the other treatments without glucose addition, but decreased respiration with glucose addition (Fig. 1A). This suggests that the addition of trophic levels beyond the microbe only treatment resulted in similar amounts of cumulative respiration. Mikola and Setälä (1998) similarly found that additional trophic levels beyond a microbe only control did not exhibit differences in respiration. Yet closer examination of our results suggests that the similarity in cumulative respiration could ultimately be driven by different underlying factors – a combination of increasing microbial biomass and decreasing mass specific respiration – that are related to increasing trophic levels (Fig. 1B and C).

The presence of the predator was associated with greater active microbial biomass and lower microbial mass specific respiration, regardless of glucose treatment. Additionally, a marginally significant

interaction was noted for mass-specific respiration, where the addition of glucose tended to be associated with lower mass-specific respiration (higher efficiency) in the microbe only treatment (Fig. 1C). The observed increase in microbial efficiency with the addition of glucose is predicted by the microbial efficiency-matrix stabilization framework (Cotrufo et al., 2013). Specifically, this framework suggests that labile plant constituents are the main resource used in microbial production because they are used most efficiently. However, our results indicate that the increase in efficiency associated with labile inputs may only occur under conditions where higher trophic levels are absent (i.e. only microbes are present), a circumstance unlikely to occur in any soils. Under circumstances where higher trophic levels are present a combination of factors may be at play, including the release of microbial nutrients and increased basal respiration caused by faunal grazing (Bonkowski et al., 2000; Bonkowski, 2004; Rousk, 2016).

Our results indicate that trophic level, particularly predator presence, may exert a stronger influence on microbial efficiency regardless of the addition of a labile substrate. The importance of predation as a driver of ecosystem processes has a long history in aboveground ecosystems (Hairston et al., 1960). The role of predation in belowground ecosystems, however, has often been difficult to disentangle and largely within the purview of detrital inputs (but see Moore et al., 2003). There is a growing realization that the role of predators must be considered within the context of labile inputs (Buchkowski, 2016; Hawlena and Zaguri, 2016). When belowground predators are considered with regards to these labile inputs, then it has been observed that they can affect ecosystem processes. For instance, increased abundance of predatory mites was associated, in situ, with decreased respiration, and increased retention of glucose-derived C (Strickland et al., 2012). Here we suggest a potential mechanism by which belowground predators can influence ecosystem C processes. Predator presence leads to increased microbial biomass and decreased mass-specific respiration. We predict that this is likely mediated by decreased microbivory and/or shifts in microbivore physiology – i.e. consumptive and/or non-consumptive effects, respectively (Guariento et al., 2015).

The fear treatment often yielded results that were intermediate for

those observed for either the predator or collembola and microbe treatments. This may suggest that non-consumptive effects do influence microbial biomass and efficiency but that consumptive effects dominate. However, the design of our study may not have allowed us to clearly disentangle non-consumptive from consumptive effects. That is the predator was placed in a 'cage' on the soil surface which may have reduced the exposure of the prey to the predator. Studies aimed at examining non-consumptive effects often inhibit predator feeding but do not limit direct contact with the prey (e.g., [Hawlena and Schmitz, 2010b](#)). Future studies aimed at examining non-consumptive effects in soil food-webs should attempt the same, especially given the widespread importance of non-consumptive effects which have been primarily observed in aboveground systems ([Sitvarin and Rypstra, 2014](#)). Regardless, particulate organic matter (POM) C:N ratios lend support to the potential importance of non-consumptive effects in soil food webs. We observed lower POM C:N ratios for both the fear and predator treatments ([Fig. 1D](#)). One explanation for this is a shift in prey physiology whereby predator presence leads to increased N excretion in prey feces subsequently lowering fecal C:N ratios ([Hawlena and Schmitz, 2010b](#)). Collembola feces is likely to be associated with the soil POM pool and as such a shift in fecal C:N may account for the lower POM C:N ratio we observe when the predator is present. This indicates that belowground predators may initiate a cascade of effects leading to increased soil nutrient availability which may ultimately influence plant growth and community composition ([Thakur et al., 2015](#)).

4.2. Effect of trophic level on microbial community composition and function

We observed that trophic level did influence soil microbial community composition. For bacteria, trophic level interacted with glucose addition to affect community composition ([Fig. 2](#)). Specifically, we observed that with or without addition of glucose the predator treatment was significantly different from the microbe treatment, and with glucose addition the fear treatment was also significantly different from the microbe treatment. These results differ from those observed by [Mikola and Setälä, \(1998\)](#), who found that bacterial composition (determined via PLFA) was unaffected by increasing trophic level. These differences associated with predator presence, in part, can be attributed to a greater abundance of families Acidothermaceae without glucose addition and Chitinophagaceae with glucose addition. Both groups are associated with the degradation of complex substrates such as chitin ([Bailey et al., 2013](#); [Smith et al., 2016](#); [Bárta et al., 2017](#)). Chitin itself may increase due to collembola mortality when predators are present. Additionally, given the fungivorous nature of collembola ([Crowther et al., 2011](#)), our results question the distinction between fungal and bacterial energy channels ([Hunt et al., 1987](#); [Bradford, 2016](#)). That is, while collembola may primarily feed on fungi, consumptive and non-consumptive effects of predators, such as shifts in POM C:N or total inputs that influence soil bacteria, may muddy this channelization.

For fungal community composition ([Fig. 3](#)) only a marginally significant effect of trophic level was observed. This effect on the fungal community was driven by differences between the microbe treatment and the collembola treatment. This difference is likely accounted for by the fact that the collembola species used in this study is considered a fungivore ([Fountain and Hopkin, 2005](#)). This marginal difference in fungal community composition may be accounted for by a decrease in Agaricomycetes within the phylum Basidiomycota ([Fig. 3B](#)). This decrease in Basidiomycota caused by a fungivore is similar to that observed by [Crowther et al., \(2015\)](#). However, our results suggest the potential for predators to serve as a release from fungivory for the Basidiomycota. This is of particular interest since fungivores have been shown to mediate ecosystem feedbacks to climate change ([Crowther et al., 2015](#)). Our results indicate that the presence of predators that feed on fungivores could release Basidiomycota from fungivory, thus dimensioning or negating any damping effect fungivores have on

ecosystem feedbacks to climate change.

For microbial community function determined via extracellular enzyme activity ([Fig. 4](#)), we observed an interaction between trophic level and glucose addition in some ways similar to the changes observed for bacterial community composition. One difference though was the observation that without the addition of glucose, treatments containing the predator had different enzyme profiles compared to the collembola treatment. Based on the response of specific enzymes this difference may be accounted for by the greater NAG activity, as well as, total hydrolytic enzyme activity associated with the fear, predator, and microbe only treatments. This may suggest that the presence of predators (both directly and indirectly) release soil microbes from microbivory effects associated with collembola, allowing for increased microbial allocation to extracellular enzyme production. Similar results, at least between the presence and absence of a microbivore, have been observed for enzyme activity associated with wood decomposing fungi ([Crowther et al., 2015](#)). However, our results indicate that this response may be dependent on C availability. Specifically, with glucose addition, we observed that the higher trophic levels all had similar enzyme profiles and were distinct from the microbe only treatment. These differences in enzyme profiles may be accounted for by lower enzyme activity associated with the microbe only treatment compared to the other treatments for all but AP activity. This greater relative allocation towards AP in the microbe only treatment may be due to increased microbial allocation towards growth ([Elser et al., 2003](#)) with the combination of glucose addition and release from microbivory. The increase in C and N acquiring enzyme activity associated with higher trophic level compared to the microbe only treatment may be due to increased cycling rates and/or investment in anti-microbivory defenses by the soil microbial community ([Jiang et al., 2017](#)).

5. Conclusions

The driving role of predators as it relates to ecosystem processes has been highlighted across multiple systems, but their role in soils has often been underrecognized. The rationale for this is that these belowground systems are dominated by bottom-up controls ([Moore et al., 2003](#)). However, this often fails to take into account both the direct and indirect effects predators can have on belowground systems ([Hawlena and Zaguri, 2016](#)). Although, it has been shown that top-down effects become increasingly important once resource limitation is alleviated ([Crowther et al., 2015](#)). Here we expand on that work to show that predators can influence soil microbial processes leading to an increase in microbial biomass and microbial efficiency, shifts in the stoichiometry of the soil POM pool, and change microbial community composition and enzyme activity. All of these changes occurred even though total soil respiration was unchanged across treatments containing trophic levels above microbes, indicating that bulk soil process measurements are not always sensitive to changes in trophic levels. However, predator effects can manifest through the soil microbial community, changing the timescale at which process rates change. This study highlights the importance top-down and bottom up controls in soil ecosystems and, demonstrates the importance of looking beyond microbivores when studying top-down ecosystem effects.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank the researchers at the University of Idaho's Genomics Resource Core for their work on the microbial sequencing. Microbial sequencing data collection and analyses performed by the IBEST

Genomics Resources Core at the University of Idaho were supported in part by NIH COBRE grant P30GM103324. We thank Ashley Irons for her invaluable help setting up this experiment and monitoring soil respiration. S.G. McBride, J.M. Lucas, and M. S. Strickland were partially supported by National Science Foundation grants (1556753 and 1832888).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.soilbio.2020.107756>.

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