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Soil biological responses to C, N and P fertilization in a polar desert of Antarctica

Becky A. Ball[a,*], Byron J. Adams[b], J.E. Barrett[c], Diana H. Wall[d], Ross A. Virginia[e]

[a] School of Mathematical and Natural Sciences, Arizona State University at the West Campus, Glendale, AZ, 85306, USA
[b] Department of Biology, Evolutionary Ecology Laboratories, Monte L. Bean Museum, Brigham Young University, Provo, UT, 84602, USA
[c] Biological Sciences, Virginia Tech, Blacksburg, VA, USA
[d] Department of Biology and School of Global Environmental Sustainability, Colorado State University, Fort Collins, CO, 80523, USA
[e] Environmental Studies Program, Dartmouth College, Hanover, NH, 03755, USA

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ABSTRACT

In the polar desert ecosystem of the McMurdo Dry Valleys of Antarctica, biology is constrained by available liquid water, low temperatures, as well as the availability of organic matter and nutrient elements. These soil ecosystems are climate-sensitive, where projected future warming may have profound effects on biological communities and biogeochemical cycling. Warmer temperatures will mobilize meltwater from permafrost and glaciers, may increase precipitation and may be accompanied by pulses of nutrient availability. Enhanced water and nutrient availability have the potential to greatly influence desert soil biology and ecosystem processes. The objectives of this 5-year study were to determine which nutrient elements (C, N, P) are most limiting to dry valley soil communities and whether landscape history (i.e., *in situ* soil type and stoichiometry) influences soil community response to nutrient additions. After 3 years of no noticeable response, soil CO_2 flux was significantly higher under addition of C + N than the other treatments, regardless of *in situ* soil stoichiometry, but microbial biomass and invertebrate abundance were variable and not influenced in the same manner. A stable isotope incubation suggests that fertilization increases C and N mineralization from organic matter via stimulating microbial activity, with loss of both the applied treatments as well *in situ* C and N. However, these responses are relatively short-lived, suggesting long-term impacts on C and N cycling would only occur if meltwater and nutrient pulses are sustained over time, a scenario that is increasingly likely for the dry valleys.

1. Introduction

Desert ecosystems are close to the physical limitations of life. Biology is constrained by available liquid water, as well as the availability of organic matter and nutrient elements that are often at limiting concentrations or in proportions outside the necessary stoichiometric ratios for balanced growth (Virginia et al., 1982; Fountain et al., 1999; Neff et al., 2000). This is especially true for polar deserts (Convey, 1996; Barrett et al., 2007), where low annual temperatures pose an additional constraint (Fountain et al., 1999). The McMurdo Dry Valleys, a polar desert region in South Victoria Land, Antarctica, is one of the coldest and driest deserts on Earth, with annual average temperatures of −20 °C and only 3–50 mm precipitation per year, water equivalent (Fountain et al., 1999, 2010). Despite the physical constraints on biological and ecosystem processes, biological activity occurs during the summer when air temperatures are at or near the freezing point, given that soil temperatures during summer are often several degrees warmer than air temperature (Simmons et al., 2009; Lacelle et al., 2016) to maintain temperatures at which these cold-adapted taxa are capable of functioning (e.g., Hopkins et al., 2006; Ball and Virginia, 2015). Because small fluctuations in temperature can therefore influence the amount of meltwater generation (Ebnet et al., 2005), the McMurdo Dry Valleys are a climate-sensitive system, where small changes in temperature can greatly alter the hydrologic regime and therefore biological and ecosystem processes (Fountain et al., 1999; Lyons et al., 2005; Barrett et al., 2008a; Ball and Virginia, 2012). The McMurdo Dry Valleys may therefore be near a tipping point (Wall, 2007), where the projected changes in climate (Chapman and Walsh, 2007; Steig et al., 2009; Walsh, 2009) could have profound effects on biological communities and biogeochemical cycling, especially if stored sources of water as ice or permafrost thaw (Fountain et al., 2016).

In high-latitude deserts, meltwater pulses may be accompanied by increases in nutrient availability (Robinson et al., 1998; Barrett et al., 2008b; Ball and Virginia, 2012). The resulting release from nutrient

* Corresponding author. School of Mathematical and Natural Sciences, Arizona State University at the West Campus, 4701 W. Thunderbird Rd., Glendale, AZ, 85306, USA.
E-mail address: becky.ball@asu.edu (B.A. Ball).

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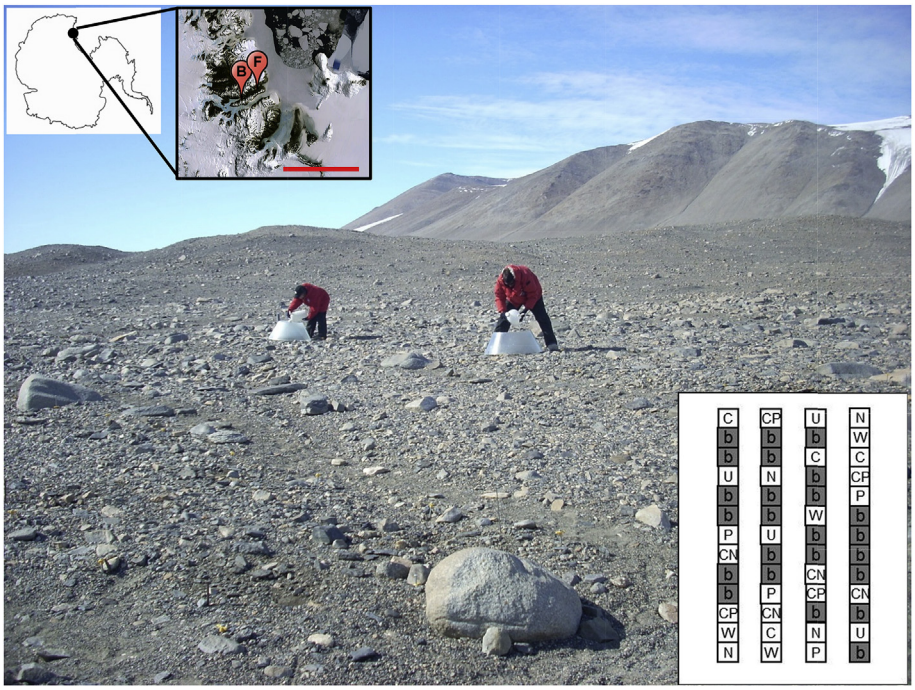

Fig. 1. Diagram of the study site, including the location of Taylor Valley, Antarctica (inset at upper left), application of annual treatments at one of the field sites (photo), and an example of four of the 8 replicate blocks at each site (inset at lower right). Satellite imagery in the inset is courtesy of NASA, available through Google Earth; the red bar represents approx. 100 km. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

limitations has the potential to greatly influence desert soil biology and the ecosystem processes in which they participate (Gebauer and Ehleringer, 2000; Austin et al., 2004; Barrett et al., 2008b). The limitation (and frequently co-limitation) of primary production by N and P is widespread (Elser et al., 2007; Harpole et al., 2011). Studies also show that nutrient additions can influence heterotrophic soil communities and processes, but results are inconsistent across studies, with some studies showing implications for belowground communities and processes and others showing no response (Ramirez et al., 2010; Lamb et al., 2011; Chen et al., 2015; Yang et al., 2017). However, while research has addressed consequences of increased nutrient availability for arid, temperate ecosystems (e.g., Liu and Crowley, 2009; Rao et al., 2009; Hall et al., 2011), as well as other polar ecosystems in the Arctic tundra (e.g., Churchland et al., 2010; Lamb et al., 2011; Wardle et al., 2013), very few studies have investigated multi-year impacts of altered water and nutrient availability in polar deserts such as the McMurdo Dry Valleys of Antarctica (with one known field manipulation in this region described by Hopkins et al., 2008). The low primary production in this region limits contemporary carbon (C) fixation into terrestrial biogeochemical cycles, and as a result these soil ecosystems are thought to be primarily C-limited (Hopkins et al., 2008; Dennis et al., 2012), with at least a portion of the C utilized by soil communities coming from 'legacy carbon' originating from ancient lake sediments rather than contemporary C-fixation (Burkins et al., 2000; Hopkins et al., 2006). It is possible that an increase in N or P mineralization with water pulses will not initially influence biological communities as noticeably as other ecosystems, without first an increase in organic matter production that could result from the stimulation of net primary production. As such, water and nutrient pulses could stimulate overall biogeochemical cycling of C, N, and P in the dry valleys.

In the McMurdo Dry Valleys, soil stoichiometry is determined by abiotic factors such as geologic legacies [e.g. eolian inputs (Lancaster, 2002), glacial till provenance (Heindel et al., 2017), surface chemistry (Heindel et al., 2018), and atmospheric deposition] and hydrologic linkages (transport of nutrients), which then influence the biological communities and ecosystem processes across the landscape (Moorhead et al., 1999; Barrett et al., 2007). For example, species distribution in soil are related to water availability, temperature, and soil chemistry, particularly salinity, where high levels of salt-forming ions such as NO_3^- [a noted legacy result of geology and age that differentiates basins (Magalhães et al., 2012; Czechowski et al., 2016; Lyons et al., 2016)], negatively influence dry valley biota via osmotic imbalance and therefore toxicity (Courtright et al., 2001; Nkem et al., 2006; Poage et al., 2008). Climate variability plays a role in landscape development that determine these geologic legacies, suggesting that future climate warming will play a role in dry valley biogeochemistry. Such chemical and biological differences among locations may influence how the ecosystems respond to changes in nutrient availability that are predicted to accompany climate change.

The objectives of this study were to determine whether the elements most limiting to Antarctic Dry Valley heterotrophic soil communities differ in contemporary edaphic properties such as native N and P content, likely as a result of their different landscape histories (largely N deposition, P weathering). We studied two different locations within one valley that naturally differ in biogeochemical properties, geologic legacies, and biotic potential, allowing us to examine how soils differing in these properties respond to annual water pulses and resource availability. We hypothesized that C will be most limiting, thus C additions will increase soil CO_2 flux (a proxy for soil respiration) and biomass of soil communities, while nutrient elements (N and P) alone will not stimulate biotic activity. However based on previous experiments (Nkem et al., 2006; Poage et al., 2008), we predicted that elevated levels of NO_3^- will increase nematode mortality. We further hypothesized that biogeochemical differences resulting from landscape history influence the response of soil communities to nutrient additions. Soils high in native N, but low in native C and P content will respond to C and CP additions, but not to N additions, and soils high in native soil P content will respond to C and possibly CN additions, but not to CP additions.

2. Methods

2.1. Study site

The study was conducted in two lake basins in Taylor Valley in the McMurdo Dry Valleys, Victoria Land, Antarctica (Fig. 1). Fryxell basin (77°36.5′S, 163°14.9′E) soils are Typic Haploturbels occurring on Ross Sea drift (late-Quaternary) that contain ice-cemented permafrost and

Table 1
Soil properties at the study site in the two lake basins of the negative controls (unamended soils), averaged across the years of the study. Values are an average of all 32 plots (8 replicates x 4 years) ± standard error.

	Fryxell basin	Bonney basin
Scottnema lindsayae abundance (# kg^{-1} dry soil)	1918.1 ± 138.8	511.5 ± 138.2
Eudorylaimus sp. abundance (# kg^{-1} dry soil)	15.77 ± 3.8	12.8 ± 3.6
Tardigrade abundance (# kg^{-1} dry soil)	0.00 ± 0.00	0.32 ± 0.32
Total invertebrate abundance (# kg^{-1} dry soil)	1933.9 ± 139.3	525.3 ± 141.1
Soil water content (% g g^{-1} dry soil)	2.0 ± 0.2	1.3 ± 0.2
Soil NH_4^+–N (μg g^{-1} dry soil)	0.12 ± 0.04	0.15 ± 0.03
Soil NO_2 + NO_3-N (μg g^{-1} dry soil)	1.71 ± 0.39	1.79 ± 0.55
Soil PO_4^{3-}P (μg g^{-1} dry soil)	3.26 ± 0.31	0.36 ± 0.04
Total soil C (%)	0.13 ± 0.00	0.08 ± 0.00
Organic soil C (%)	0.024 ± 0.009	0.009 ± 0.003
Total soil N (%)	0.015 ± 0.009	0.005 ± 0.003
pH	9.53 ± 0.16	8.89 ± 0.13
Electrical conductivity (μS cm^{-1})	262.7 ± 42.4	222.9 ± 22.3
Microbial carbon (μg g^{-1} dry soil)	11.79 ± 1.66	7.27 ± 0.77
Soil respiration (μmol C m^{-2} s^{-1})	0.14 ± 0.06	0.07 ± 0.02

are strongly cryoturbated (Bockheim and McLeod, 2008; Bockheim et al., 2008). Bonney basin (77°43.6′S, 162°18.8′E) soils are Typic Anhyorthels occurring on Taylor III drift (mid-to early-Quaternary) in areas of dry permafrost. In both basins, soils are poorly-developed and coarse (typically 95–99% sand), dry (largely < 1–5% moisture), high in salt content and pH, and low in organic matter compared to other ecosystems (typically 0.1–0.3 mg organic C g $soil^{-1}$), with a relatively shallow active layer (10–70 cm) underlain by 200–600 m of perennial permafrost (Campbell and Claridge, 1987; Campbell et al., 1997; Burkins et al., 2000; Bockheim et al., 2007). Biology is limited to low-biomass soil communities that are dominated by surprisingly diverse microbial communities with a few invertebrate taxa (Cary et al., 2010). The most abundant and widely distributed invertebrate is a microbial-feeding nematode, *Scottnema lindsayae* Timm, 1971, which is often the sole metazoan invertebrate species in soils (Freckman and Virginia, 1997; Barrett et al., 2004; Adams et al., 2007). An additional nematode, *Eudorylaimus* sp., has a more limited distribution, low abundance, and co-occurs with *S. lindsayae* (along with *Plectus* spp.) in suitable soil habitats (Treonis et al., 1999; Adams et al., 2006, 2014). However, the basins significantly differ in basic soil properties (Table 1; Burkins et al., 2001; Barrett et al., 2002; Barrett et al., 2004; Bate et al., 2008; Simmons et al., 2009; Heindel et al., 2018), causing them to support different communities and activities (Barrett et al., 2006). Notably, soil C content, as well as extractable forms of mineral N and P, differs between the two basins, where Fryxell Basin soils tend to have higher C and P > N, and Bonney Basin soils tend to have lower C and N ≥ P. Both basins have a rather low C:N ratio, of 1:9 in Fryxell and 1:16 in Bonney (or nearly 1:2 in both locations, if considering only organic C), suggesting C limitation.

2.2. Field experiment

In the 2006–2007 austral summer field season, a long-term field manipulation experiment was established on soils free of visible soil microbial or plant crusts. At both the Fryxell and Bonney basins, we delineated 8 replicate rows of 13 adjacent 1 m × 1 m plots (Fig. 1). From each row of 13 plots, 7 were selected to be used in the experiment, and the remaining 6 were left blank to avoid boulders, polygon cracks, or other features that would prevent the square from serving as an appropriate replicate. Thus, we had 56 experimental plots (8 replicates of 7 treatments) at each basin. To the 7 plots in each row, we randomly assigned one of 7 treatments: carbon (C), nitrogen (N), phosphorus (P), carbon and nitrogen (CN), carbon and phosphorus (CP), water only positive control (W), and an un-amended negative control (U). Nutrient treatments were applied as aqueous solutions at a rate that brought the top 10 cm of soil to field capacity (following application rates of other experiments; Burkins et al., 2001). C was added at a rate of 15.27 g C m^{-2} as mannitol (a simple alcohol-sugar derivative of mannose, a common algal sugar). N and P were added in molar ratios of 10 C:N and 100 C:P, with N at a rate of 2.69 g N m^{-2} as NH_4NO_3 and P at a rate of 0.37 g P m^{-2} as $Na_3PO_4{\cdot}12H_2O$. When treatments were applied to each plot, a plastic open-top chamber (cone) was centered over the plot with an area of 0.44 m^2 (Fig. 1) to provide an outline for treatment application. 5.6 L of treatment solution (either water or nutrient solution at the appropriate concentration) was sprinkled in a circular pattern to cover the entirety of the soil within the cone, pouring at a steady rate that allowed percolation and no surface runoff. The cone was then removed from the plot.

Treatments were applied annually for 6 years. Prior to treatment in years 1, 3 and 5, soil surface samples (0–10 cm) were collected from the field using a sterile plastic scoop and plastic bag, then transported to the Crary Lab at McMurdo Station. Within two weeks following treatment application in all years, soil CO_2 flux was measured in the field on three or four of the replicate plots using a Li-COR 8100 (LI-COR Biosciences, Lincoln, NE) by fitting a soil respiration chamber overtop a 10-cm diameter PVC ring that had been inserted into the soil at least 1 h prior to measurement, as per previous protocols (Ball and Levy, 2015; Ball and Virginia, 2015). The height of the collar aboveground (offset) was measured for calculation of volume of the headspace in each PVC ring. Once the sealed chamber was placed over the collar, CO_2 flux was recorded for 60 s after a 10 s deadband. The raw data for each individual measurement were reviewed during measurement, and those visually determined to have high noise were disregarded and the plot re-measured. The flux measured using a linear fit was recorded, as is recommended by LI-COR for low flux levels. In low biomass Antarctic soils physical factors, driven by change in temperature and solubility in water films, can dominate over biological sources of soil CO_2 flux (Parsons et al., 2004; Ball et al., 2009; Shanhun et al., 2012), so CO_2 flux measurements were made during the warmest part of the day at the peak of summer (January), when biological signals would be strongest. All plots at each basin were sampled within a few hours, and all treatments within a replicate block were measured before moving on to the next replicate block, to ensure that changes in temperature and weather are not responsible for any treatment effects measured, and the random assignment of treatments to plots within each block ensures that treatment effects are not the result of *in situ* physical differences. Thus, differences among treatments are highly unlikely to be the result of differences in physical factors influencing CO_2 flux and instead be the result of altered biological activity. Soil temperature and moisture in the nearby soil outside the ring was recorded at each measurement using probes [Delta theta probe (Delta-T Devices) and thermistor probe] attached to the LI-COR 8100.

In the lab, soils were subsampled for invertebrate analysis in a laminar flow hood. Approximately 100 g of fresh soil was extracted using a modified sugar-centrifugation technique (Freckman and Virginia, 1993) for enumeration of invertebrates under an inverted microscope. Abundance of live and total (live + dead) nematodes were determined for each species present, as well as tardigrades and rotifers. Microbial C and N were measured using the chloroform fumigation extraction technique by extracting 35 g of soil from each sample in 70 ml of 0.5 M K_2SO_4 (Horwath and Paul, 1994). Extracts were shaken at 200 rpm for 30 min, centrifuged at 25000 × *g*, then poured through 0.45 μm nylon filters and acidified with 3 ml 6N HCl. A duplicate 35-g subsample was placed in a vacuum desiccator and fumigated with ethanol-free chloroform for 120 h. After fumigation, soils were extracted as described above. All extracts were frozen prior to analysis on a Shimadzu TOC analyzer for DOC and DIN.

To measure soil chemistry, soil was sieved to < 2 mm. Soil water content was estimated from a 20 g soil sample that was dried at 105 °C

for 24 h. Soil pH was measured using a 1:2 solution of soil:di-H_2O using a pH meter (VWR Scientific model 8015, VWR Scientific, West Chester, PA). Conductivity (EC) was measured using a 1:5 solution of soil:di-H_2O using a conductivity meter (Orion model #160, Orion Research Incorporated, Boston, MA; Barrett et al., 2004). Total N and organic soil C were determined using a Carlo Erba Elemental 1500 Analyzer (Milan, Italy; Barrett et al., 2004), with organic C samples having been acidified with HCl. Extractable phosphate (PO_4—P) was measured by extracting 10 ± 0.5 g soil in 50 ml 0.5 M $NaHCO_3$ at pH 8.5. Samples were filtered to remove soil, acidified with 3 ml of 6 N HCl, then allowed to degas prior to being frozen until run on a Lachat Autoanalyzer (Barrett et al., 2007). Extractable inorganic N (NO_3 + NO_2-N and NH_4—N) was measured by extracting 20 ± 0.5 g soil in 50 ml 2 M KCl, filtered, then frozen until run on a Lachat Autoanalyzer (Barrett et al., 2007).

2.3. Laboratory incubation

A stable isotope tracer incubation was performed using the U, C, and CN treatment soils collected from both basins in the 2009–10 field season (Year 3). From 3 randomly-selected replicate plots, 10 g fresh weight soil were weighed into each of 3 separate Exetainer glass vials (12 ml, 10 × 1.5 cm; Labco, High Wycombe, Buckinghamshire, England) from the U and C treatments (3 plots × 3 vials = 9 vials of U and 9 vials of C per basin), and two sets of 3 separate vials from the CN treatment (3 plots × 3 vials x 2 sets = 18 vials of CN per basin, twice as many as they are used for two different treatments). Vials were allowed to sit at room temperature for 2 d to allow for the labile flush of dead microbial cells (Bate, 2007), after which one of the 3 replicate vials from each sample was processed for initial soil conditions (Appendix 1). To the 2 remaining replicate vials of each sample, a new treatment was applied at the same rate as field applications using isotopically labeled compounds comprised of a mixture of regular and labeled material to 1 At%, with $\delta^{15}N$ of 2000‰ and $\delta^{13}C$ of 45‰. The U treatment received 750 µl of de-ionized water. The C's received 750 µl of a 3 µg/µl ^{13}C-mannitol solution. The CN received 750 µl of the labeled mannitol solution that also contained 0.76 µg/µl ^{15}N-KNO_3, and 0.40 µg/µl ^{15}N-NH_4Cl. The additional set of vials containing CN soils received 750 µl of the same CN treatment with 0.35 µg/µl Na_3PO_4 (unlabeled). Because the field experiment did not indicate an influence of P or CP alone, we only test here for an effect of P when added alongside the CN treatment. The treatment solutions were verified by drying 200 µl of each in tin capsules for isotopic analysis on a mass spec (see below). The total weight of the vial after treatment addition was measured, and periodically DI-H_2O was added to the vials to bring them back to this weight, so as to maintain a steady moisture content.

Jars were incubated in the laboratory at room temperature (approximately 22 °C), to examine the soil community's potential to utilize carbon and denitrify N in response to the nutrient treatments. By freeing the biology from the constraints of the cold natural environment, we increased the potential to measure small short-term response to treatment alone. At regular sampling points over the course of 23 d, vials were capped, with one vial being used to measure CO_2 flux (respiration) and the other used to measure N_2O accumulation (denitrification).

To measure respiration, the lids were screwed tightly onto each vial, plus three empty vials containing ambient atmospheric conditions. CO_2 was allowed to accumulate in the headspace. After 24 h, a 5-ml sample of headspace was injected into a LI-COR 6262 (LI-COR Inc., Lincoln, NE), and the accumulated CO_2 concentration was calculated as the difference between the sample's CO_2 concentration minus atmospheric CO_2, then used to calculate net C mineralization rate using the equation

$$(AW_C * [CO_2] *V) / (R * T) / (S * d) \quad (1)$$

where AW_C is the atomic weight of C (12 g mol^{-1}), V is the headspace volume, R is the gas constant (0.082 L ppm K^{-1} mol^{-1}), T is room temperature (298 K), S is soil mass, and d is days of CO_2 accumulation. Additionally, to identify treatment-induced changes in the pool of C being mineralized and respired, vials were recapped, allowed to accumulate in CO_2 for 48 h, and a subsample of headspace was removed for measurement of $\delta^{13}C$ on a stable isotope mass spectrometer (Thermo Finnigan DeltaPLUS XL interfaced with GasBench II, ThermoFisher Scientific, Waltham, MA; Bate, 2007).

Denitrification potential was measured using the acetylene block technique. Though the caveats associated with the acetylene block method prevent us from making strong conclusions about actual rates of denitrification (Groffman et al., 2006; Qin et al., 2013), it is a useful method for measuring the potential for denitrification to occur and differences among treatments. Acetylene (C_2H_2) was made by adding 20 ml di-H_2O to one CaC_2 rock in a 125-ml flask, allowing the flask to degas until the flasks' atmosphere was filled with C_2H_2, then adding a cork with a needle to collect the C_2H_2 gas in a tedlar bag. Prior to each sampling, vials were capped and flushed for 45 s with N_2 gas, then 10% of the headspace was replaced with acetylene gas (Drury et al., 2008). After 72 h, a gas sample was removed for measurement of N_2O concentration and $\delta^{15}N$ on the stable isotope mass spectrometer. Measured N_2O was used then used to calculate denitrification rate using the equations:

$$V_{N2O} = [N_2O] * (V_H + (V_w * \alpha))/1000 \quad (2)$$

$$\text{Denitrification rate} = (AW_N * V_{N2O}) / (R * T) / (S * d) \quad (3)$$

where V_{N2O} is the volume of N_2O created, V_H is the headspace volume, V_w is the volume of water, α is the Bunsen absorption coefficient (0.63 ml N_2O ml H_2O^{-1}), AW_N is the atomic weight of 2 × N (28.0134 g mol^{-1}), R is the gas constant (0.082 L ppm K^{-1} mol^{-1}), T is room temperature (298 K), S is soil mass, and d is days of N_2O accumulation. A mixing model was used to determine the fraction of denitrified N that originated from the treatment (F_{trt}) as opposed to *in situ* soil N (the only two possible sources, given that the flushing removed any atmospheric N_2O prior to denitrification from soil):

$$F_{trt} = (\delta^{15}N_{hs} - \delta^{15}N_s) / (\delta^{15}N_{trt} - \delta^{15}N_s) \quad (4)$$

where N_{hs}, N_s, and N_{trt} represent the N in the headspace, soil, and treatment, respectively. The mixing model does not account for fractionation during denitrification that would reflect the preference for lighter N isotopes to be denitrified, which on average depletes the N_2O generated by approx. 18.5‰ to leave soil NO_3^- enriched with ^{15}N (Bedard-Haughn et al., 2003).

The mixing-model to determine the fraction of respired C originating from the treatment was more complex, given that headspace CO_2 was a mix of *in situ* soil C, treatment C, and atmospheric CO_2. First, the fraction of atmospheric CO_2 was calculated as the atmospheric concentration (as measured in the atmospheric vials) divided by the total concentration in the headspace, assuming no atmospheric CO_2 was absorbed into the soil, and the fraction of respired CO_2 (from both the treatment and the soil) and fraction of atmospheric CO_2 would total 1. The $\delta^{13}C$ of the respired C was calculated using a mixing model, where:

$$\delta\ ^{13}C_{respired} = [(\delta^{13}C_{hs} - \delta\ ^{13}C_{atm})/F_{respired}] + \delta\ ^{13}C_{atm} \quad (5)$$

A mixing model was then applied to the remaining fraction to determine the fraction from the treatment (Staddon, 2004):

$$F_{trt} = (\delta^{13}C_{respired} - \delta^{13}C_s) / (\delta^{13}C_{trt} - \delta^{13}C_s). \quad (6)$$

We assumed no discrimination against ^{13}C during microbial use of the substrate during respiration.

The vials used for measuring respiration were destructively sampled at d 23 and, in addition to the initial vials, were analyzed for total and organic % C and isotopic composition, as well as total % N and isotopic composition. Total and organic soil C were determined on soils ground using a sapphire mortar and pestle that had been either left unacidified

or acidified respectively, using an elemental analyzer (Costech Analytical Technologies Inc., Valencia CA) with attached mass spectrometer (Thermo Deltaplus Advantage, Thermo Scientific). Additionally, 8 g soil was removed and extracted in 20 ml 2 M KCl to measure mineral N, as described above.

2.4. Data analysis

Data were analyzed in R using a three-way Analysis of Variance (ANOVA) with time as a continuous factor. Main effects were Basin (2 levels), Treatment (7 levels), and Year (1 continuous factor). Data were log(x + 1) transformed to meet the assumptions of normality. Where Basin was significant, the analyses were re-run individually for each basin using a 2-way ANOVA with time as a continuous factor. Post-hoc Tukey tests were used to determine which treatments differed significantly.

3. Results

3.1. Field experiment

Resource treatment application increased soil NH_4^+ and NO_3^- under the N and CN treatments and soil PO_4^{3-} under the P and CP treatments (Table 2, Appendix 2). Organic C was significantly influenced by treatment, to be higher in the C treatment compared to the +N, +P and U and W controls (Fryxell basin) and higher in the CN treatment compared to the control N soils (Bonney basin) (Table 2, Fig. 2d). Treatments did not influence pH or EC (Table 2) to adversely affect habitat suitability. Additionally, the two basins significantly differed in most chemical parameters (significant Basin effect, Table 2), which influenced the treatment effects (significant Basin*Treatment interaction) for SWC, PO_4^{3-}, and NH_4^+.

Initially, treatment did not influence soil respiration (estimated as CO_2 flux). However, after year 3, respiration was significantly higher in the CN plots than the other treatments (Table 3, Fig. 2). Additionally, the Tukey test showed that C and CP, though respiring less than CN, also respired more than the untreated plots in the Bonney basin (Fig. 2c). The stimulation by CN addition diminished after year 4, and respiration appeared equivalent among treatments in year 6. Notably, SWC and its interaction with temperature significantly influenced soil CO_2 flux ($P < 0.001$ and $P = 0.004$, respectively), so local climate conditions at the time of measurement can create interannual variability in measurements. Microbial C in the soil also was only marginally affected by treatment, but it did not mirror the response of soil respiration. While CN addition stimulated respiration above all other treatments at Fryxell, microbial biomass under CN was only greater than the C treatment, and in the Bonney basin there were no differences in biomass among treatments despite the stimulated respiration (Table 3, Fig. 3a).

Both *S. lindsayae* and *Eudorylaimus* sp. were found in the soil samples, but *Eudorylaimus* sp. was the only nematode to be significantly influenced by treatment in both basins (Table 3). Again, their response did not mirror that of soil respiration, because abundance is comparatively low under CN addition where respiration was stimulated (Fig. 3b). At the Fryxell basin, their abundance was significantly lower under W, C, and P treatments compared to U and CP. At Bonney, P increased the abundance of *Eudorylaimus* sp. above densities found in N−, CN−, and CP-treated plots, despite the lack of an effect of P alone on respiration. However, *S. lindsayae*, which was not influenced by treatment, was far more abundant, and thus the response of the total invertebrate community was determined by *S. lindsayae*. Rotifer and tardigrade abundance also differed among treatments (Table 3), but this appears to result from their absence from all but a few individual samples (Fig. 3c and d). For tardigrades, this pattern was observed only in the outset of the experiment, and then they became absent from the samples after year 1 (significant effect of Treat*Year in Table 3).

3.2. Laboratory incubation

As in the field experiment, soil respiration was stimulated by the addition of C and N together, such that the CN and CNP treatments exhibited higher respiration compared to C or water alone (Table 4, Fig. 4a). This response was relatively short-lived, declining in the Fryxell basin soils after day 6 and in the Bonney soils after 2 weeks. In the Fryxell soils, the addition of CNP did not influence the soils differently than CN addition alone. In the Bonney soils, which we predicted would more likely be P limited, the CNP treatment stimulated respiration compared to the CN treatment at only the very beginning of the incubation. By day 4, respiration from the CNP treatment was no longer significantly greater than the CN treatment. In both lake basins, the C treatment increased in respiration at the last sampling date, causing C to be significantly greater than U.

Table 2
Results of a three-way Analysis of Variance comparing the effect of Basin (2 levels), Treatment (7 levels), and Year (continuous variable), as well as their interactions, on various soil chemical properties.

	Soil water content		PO_4-P		NH_4-N		NO_2+NO_3-N		pH		EC	
	F_{df}	*P*	F_{df}	*P*	F_{df}	*P*	F_{df}	*P*	F_{df}	*P*	F_{df}	*P*
Basin	$75.20_{1,420}$	**< 0.001**	$827.79_{1,420}$	**< 0.001**	$9.65_{1,419}$	**0.002**	$4.68_{1,308}$	**0.031**	$360.48_{1,420}$	**< 0.001**	$0.04_{1,420}$	0.838
Treat	$2.17_{6,420}$	**0.045**	$68.44_{6,420}$	**< 0.001**	$218.41_{6,419}$	**< 0.001**	$57.81_{6,308}$	**< 0.001**	$0.86_{6,420}$	0.522	$0.67_{6,420}$	0.677
Year	$46.69_{1,420}$	**< 0.001**	$85.09_{1,420}$	**< 0.001**	$138.40_{1,419}$	**< 0.001**	$1.34_{1,308}$	0.247	$193.38_{1,420}$	**< 0.001**	$11.03_{1,420}$	**0.001**
Basin*Treat	$3.15_{6,420}$	**0.005**	$18.25_{6,420}$	**< 0.001**	$2.91_{6,419}$	**0.009**	$0.73_{6,308}$	0.629	$1.01_{6,420}$	0.417	$1.11_{6,420}$	0.358
Basin*Year	$2.38_{1,420}$	0.124	$19.12_{1,420}$	**< 0.001**	$2.81_{1,419}$	0.094	$0.54_{1,308}$	0.461	$1.09_{1,420}$	0.297	$18.08_{1,420}$	**0.000**
Treat*Year	$0.38_{6,420}$	0.890	$32.04_{6,420}$	**< 0.001**	$73.95_{6,419}$	**< 0.001**	$19.48_{6,308}$	**< 0.001**	$0.45_{6,420}$	0.844	$1.30_{6,420}$	0.254
Basin*Treat*Year	$0.35_{6,420}$	0.911	$7.03_{6,420}$	**< 0.001**	$1.61_{6,419}$	0.142	$0.80_{6,308}$	0.571	$0.29_{6,420}$	0.942	$0.09_{6,420}$	0.997

	% Organic C		Total %N	
	F_{df}	*P*	F_{df}	*P*
Basin	$216.46_{1,244}$	**< 0.001**	$80.09_{1,244}$	**< 0.001**
Treat	$7.55_{6,244}$	**< 0.001**	$1.92_{6,244}$	0.078
Year	$0.05_{1,244}$	0.825	$294.23_{1,244}$	**< 0.001**
Basin*Treat	$1.42_{6,244}$	0.208	$1.43_{6,244}$	0.205
Basin*Year	$5.67_{1,244}$	**0.018**	$54.57_{1,244}$	**< 0.001**
Treat*Year	$0.76_{6,244}$	0.603	$1.13_{6,244}$	0.345
Basin*Treat*Year	$0.77_{6,244}$	0.597	$0.43_{6,244}$	0.860

P-values in bold are significant below $\alpha = 0.05$.

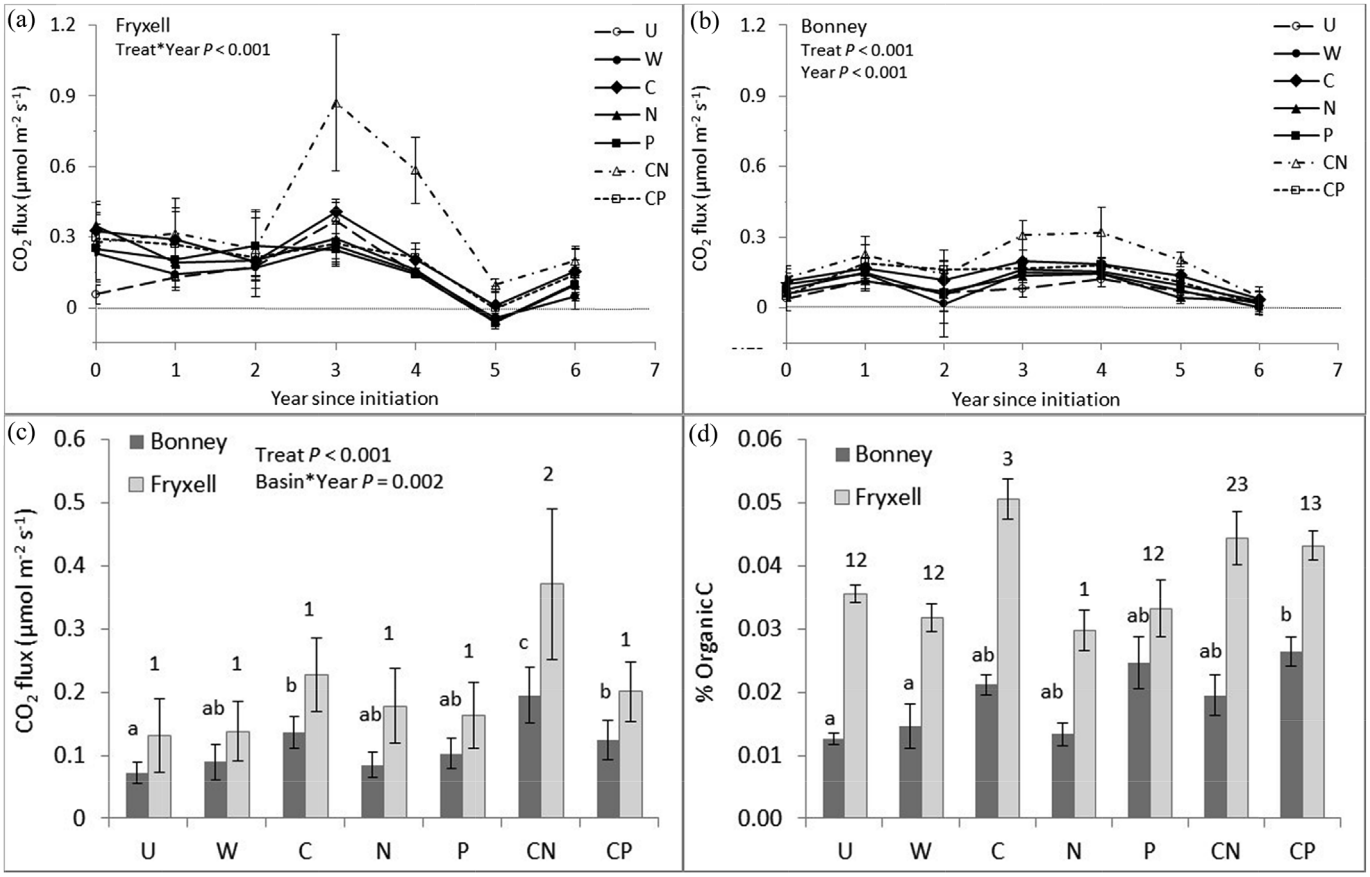

Fig. 2. Soil CO_2 flux from soils (a–b) in two lake basins receiving nutrient addition treatments over the course of 7 years, listing the highest-order significant factors identified by the ANOVA within each basin, as well as (c) average values over the 6-year study, listing the highest-order significant factors identified by the ANOVA across basins (see Table 3). (d) Soil % organic C in two lake basins. Treatments were untreated (U), water-only addition (W), carbon addition (C), nitrogen addition (N), phosphorus addition (P), carbon and nitrogen added together (CN), and carbon and phosphorus added together (CP). Letters and numbers represent differences identified by a post-hoc Tukey test run separately for each basin.

The CO_2 produced under the CN and CNP treatments was initially heavier in ^{13}C than the treatments without N (Table 4, Fig. 4b), reflecting use of the added C that declined over time. When C was added alone, the CO_2 produced was significantly heavier in ^{13}C than from untreated soils, but not as heavy as those also receiving N. By the end of the incubation, the isotopic ratios of respired CO_2 were all approximately equivalent. The mixing model indicates that the CO_2 respired was a mix of the ^{13}C-mannitol treatment and native soil C. In the Fryxell vials, respiration in the C treatments was initially only marginally from the added C, increasing to 15% later in the incubation, while respiration in the CN vials was initially 42(± 10)% from the treatment decreasing to 24(± 8)% at the end of the incubation. In the Bonney vials, the treatments comprised initially 5% of respired C increasing to 27(± 8)% by the end of the incubation under C addition, and initially 53(± 12)% in the CN and 62(± 10)% in the CNP, both decreasing to 20(± 7)% by the end of the incubation. In treatments where C was added (C, CN, CNP), organic C was heavier in ^{13}C at the end of the experiment, particularly in the Bonney basin soils (Fig. 5d). This reflects mannitol remaining in the soil, because only the C treatment (where less was used for respiration) was significantly heavier than the U control. This pattern was not reflected in total %C due to large variability (Fig. 5a–b).

Significant rates of denitrification only occurred in the soils receiving additional N (CN and CNP), and was initially greater in the Lake Fryxell basin soils than Bonney (Table 4, Fig. 4c). Higher denitrification at Fryxell corresponded with higher total soil %N in comparison to

Table 3
Results of a three-way Analysis of Variance comparing the effect of Basin (2 levels), Treatment (7 levels), and Year (continuous variable), as well as their interactions, on various soil biological communities and soil respiration.

	S. lindsayae		*Eudorylaimus* sp.		Rotifers		Tardigrades		Total invertebrates		Microbial C		Respiration	
	F_{df}	*P*	F_{df}	*P*	F_{df}	*P*	F_{df}	*P*	F_{df}	*P*	F_{df}	*P*	F_{df}	*P*
Basin	$3.11_{1,420}$	0.079	$63.42_{1,420}$	**< 0.001**	$2.08_{1,420}$	0.150	$1.12_{1,420}$	0.291	$2.49_{1,420}$	0.115	$5.58_{1,316}$	**0.019**	$60.55_{1,392}$	**< 0.001**
Treat	$0.46_{6,420}$	0.836	$2.14_{6,420}$	**0.048**	$2.21_{6,420}$	**0.041**	$2.51_{6,420}$	**0.021**	$0.52_{6,420}$	0.794	$2.12_{6,316}$	**0.051**	$16.37_{6,392}$	**< 0.001**
Year	$9.63_{1,420}$	**0.002**	$1.85_{1,420}$	0.174	$0.01_{1,420}$	0.934	$4.82_{1,420}$	**0.029**	$9.20_{1,420}$	**0.003**	$0.25_{1,316}$	0.619	$27.23_{1,392}$	**< 0.001**
Basin*Treat	$0.28_{6,420}$	0.945	$6.33_{6,420}$	**< 0.001**	$0.89_{6,420}$	0.506	$3.07_{6,420}$	**0.006**	$0.25_{6,420}$	0.961	$1.47_{6,316}$	0.189	$1.94_{6,392}$	0.074
Basin*Year	$3.17_{1,420}$	0.076	$0.02_{1,420}$	0.883	$0.04_{1,420}$	0.833	$0.93_{1,420}$	0.337	$2.90_{1,420}$	0.089	$5.60_{1,316}$	**0.019**	$10.00_{1,392}$	**0.002**
Treat*Year	$0.31_{6,420}$	0.931	$1.28_{6,420}$	0.264	$0.18_{6,420}$	0.983	$2.55_{6,420}$	**0.020**	$0.29_{6,420}$	0.942	$3.31_{6,316}$	**0.004**	$1.44_{6,392}$	0.197
Basin*Treat*Year	$0.32_{6,420}$	0.926	$0.29_{6,420}$	0.944	$0.43_{6,420}$	0.858	$3.20_{6,420}$	**0.004**	$0.32_{6,420}$	0.925	$0.70_{6,316}$	0.654	$0.89_{6,392}$	0.501

P-values in bold are significant below $\alpha = 0.05$.

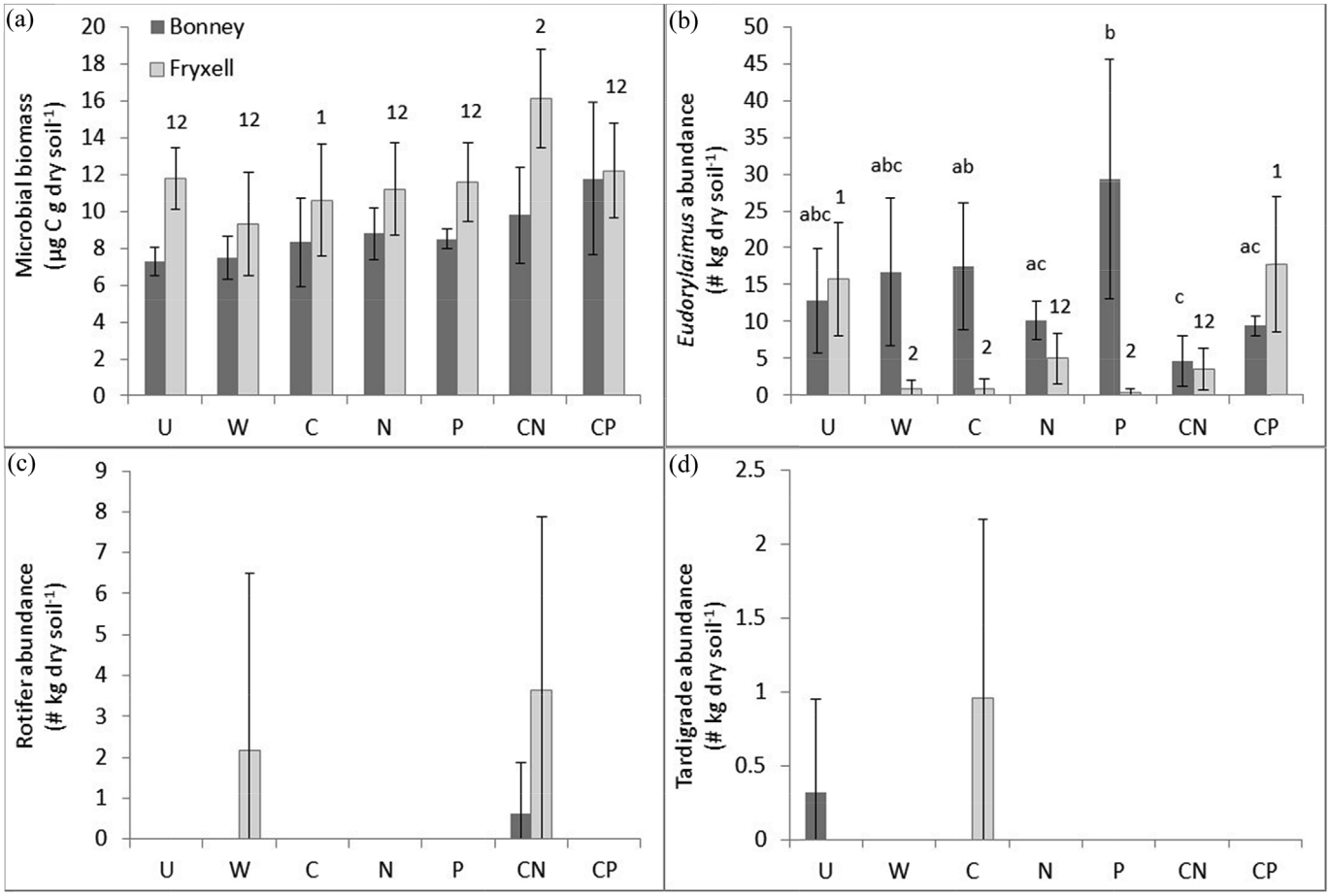

Fig. 3. (a) Microbial biomass C, and abundance of (b) *Eudorylaimus* sp., (c) rotifers, and (d) tardigrades in two lake basins receiving nutrient addition treatments averaged over the 6 years. Treatments were untreated (U), water-only addition (W), carbon addition (C), nitrogen addition (N), phosphorus addition (P), carbon and nitrogen added together (CN), and carbon and phosphorus added together (CP). Letters and numbers represent differences identified by a post-hoc Tukey test run separately for each basin.

Bonney (Fig. 5e). In the Bonney basin soils, denitrification was higher in the CNP treatment than CN alone. In both basins, the N_2O produced was isotopically heavier than atmospheric N (Fig. 4d) and the original soil (Fig. 5f), demonstrating the use of the added N treatment. However, the N_2O was not as heavy as the added N treatment (with a $\delta^{15}N$ of 1982), suggesting that N treatment stimulated denitrification of both added and *in situ* N (given that the headspace was flushed with N_2, and therefore no ambient N_2O was present). According to the mixing model, the majority of the N_2O-N collected in the headspace was from the soil. Over the course of the experiment, only 27% (± 8%) of the denitrified N is from the treatment in Fryxell vessels and 37% (± 3%) in Bonney vessels, suggesting that the remaining denitrified N was from the soil. This reflects the fractionation that would preferentially denitrify lighter *in situ* N isotopes over the applied treatment, leaving the residual heavier fraction in the soil and the lighter fraction in the headspace. Even when the ^{15}N pulse was not added (U and C treatments), soil N was heavier at the end of the experiment than the beginning (Fig. 5f). However, the CN and CNP treatments were quite heavy in ^{15}N still at the end of the experiment, suggesting that a lot of the added N still remained in the soil. This led to the CN and CNP treatments containing significantly more total %N than the untreated soils at the end of the experiment.

Table 4
Results of a three-way Analysis of Variance comparing the effect of Basin (2 levels), Treatment (4 levels), and Day (continuous variable), as well as their interactions, on levels of soil respiration and denitrification and the isotopic ratios of the gas released.

	Respiration		$\delta^{13}C$		Denitrification		$\delta^{15}N$	
	F_{df}	*P*	F_{df}	*P*	F_{df}	*P*	F_{df}	*P*
Basin	$0.04_{1,128}$	0.848	$11.46_{1,56}$	**0.001**	$11.73_{1,56}$	**0.001**	$0.12_{1,56}$	0.733
Treat	$40.83_{3,128}$	< **0.001**	$58.93_{3,56}$	< **0.001**	$6.43_{3,56}$	**0.001**	$21.69_{3,56}$	< **0.001**
Year	$17.96_{1,128}$	< **0.001**	$56.65_{1,56}$	0.790	$0.02_{1,56}$	0.878	$0.54_{1,56}$	0.466
Basin*Treat	$3.85_{3,128}$	**0.011**	$1.28_{3,56}$	0.289	$3.53_{3,56}$	**0.021**	$0.92_{3,56}$	0.437
Basin*Year	$1.62_{1,128}$	0.205	$1.13_{1,56}$	0.291	$0.28_{1,56}$	0.600	$2.38_{1,56}$	0.129
Treat*Year	$23.27_{3,128}$	< **0.001**	$16.14_{3,56}$	< **0.001**	$0.50_{3,56}$	0.685	$0.18_{3,56}$	0.909
Basin*Treat*Year	$0.46_{3,128}$	0.708	$1.92_{3,56}$	0.137	$0.29_{3,56}$	0.830	$0.84_{3,56}$	0.476

P-values in bold are significant below $\alpha = 0.05$.

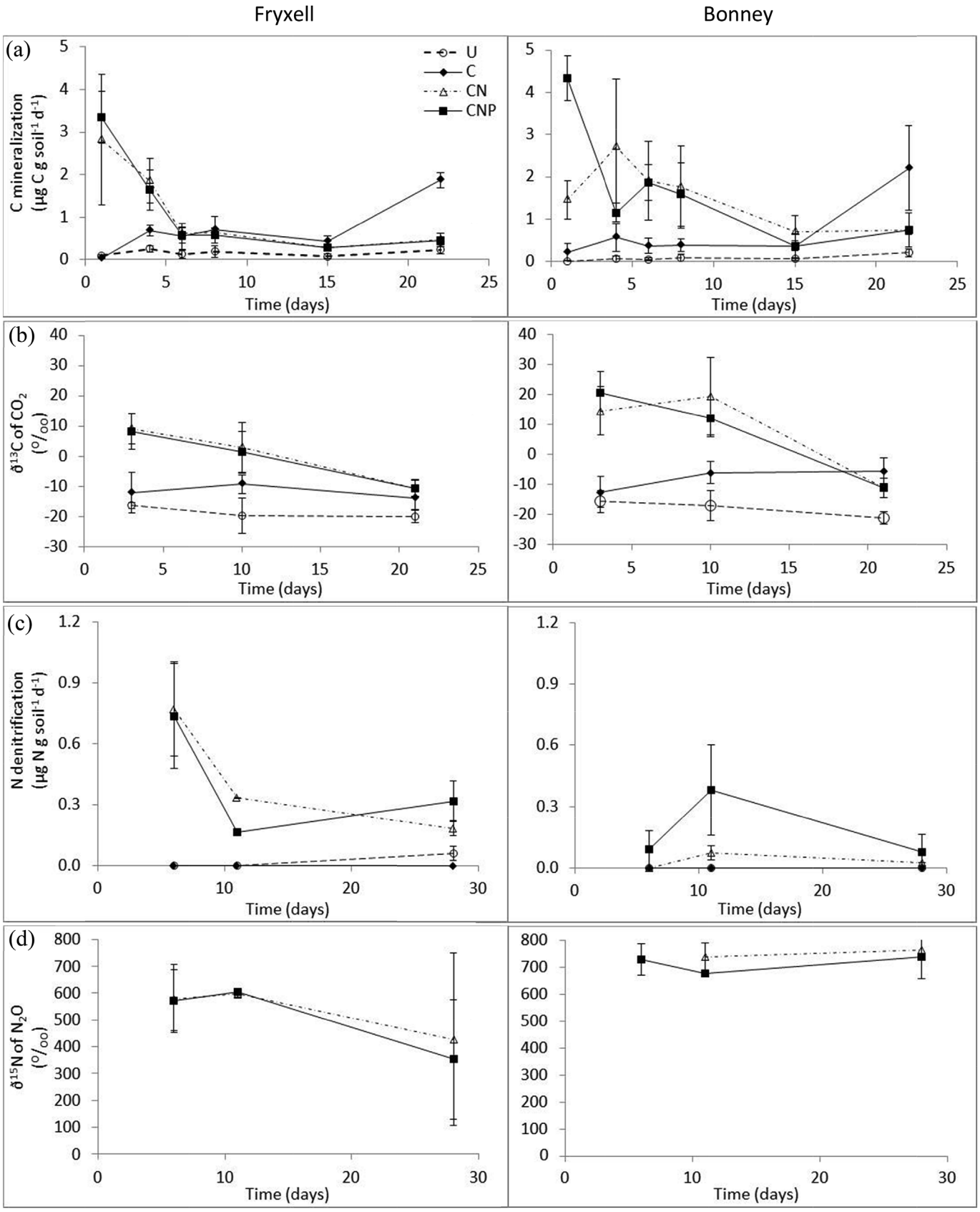

Fig. 4. Amount (a, c) and isotopic composition (b, d) of C mineralized during respiration (a, b) and N released as N_2O during denitrification (c, d) in soils from two lake basins over the course of a laboratory incubation. Soils from each basin received one of four treatments: untreated (U), ^{13}C addition (C), ^{13}C and ^{15}N addition (CN), and ^{13}C, ^{15}N, and P addition (CNP).

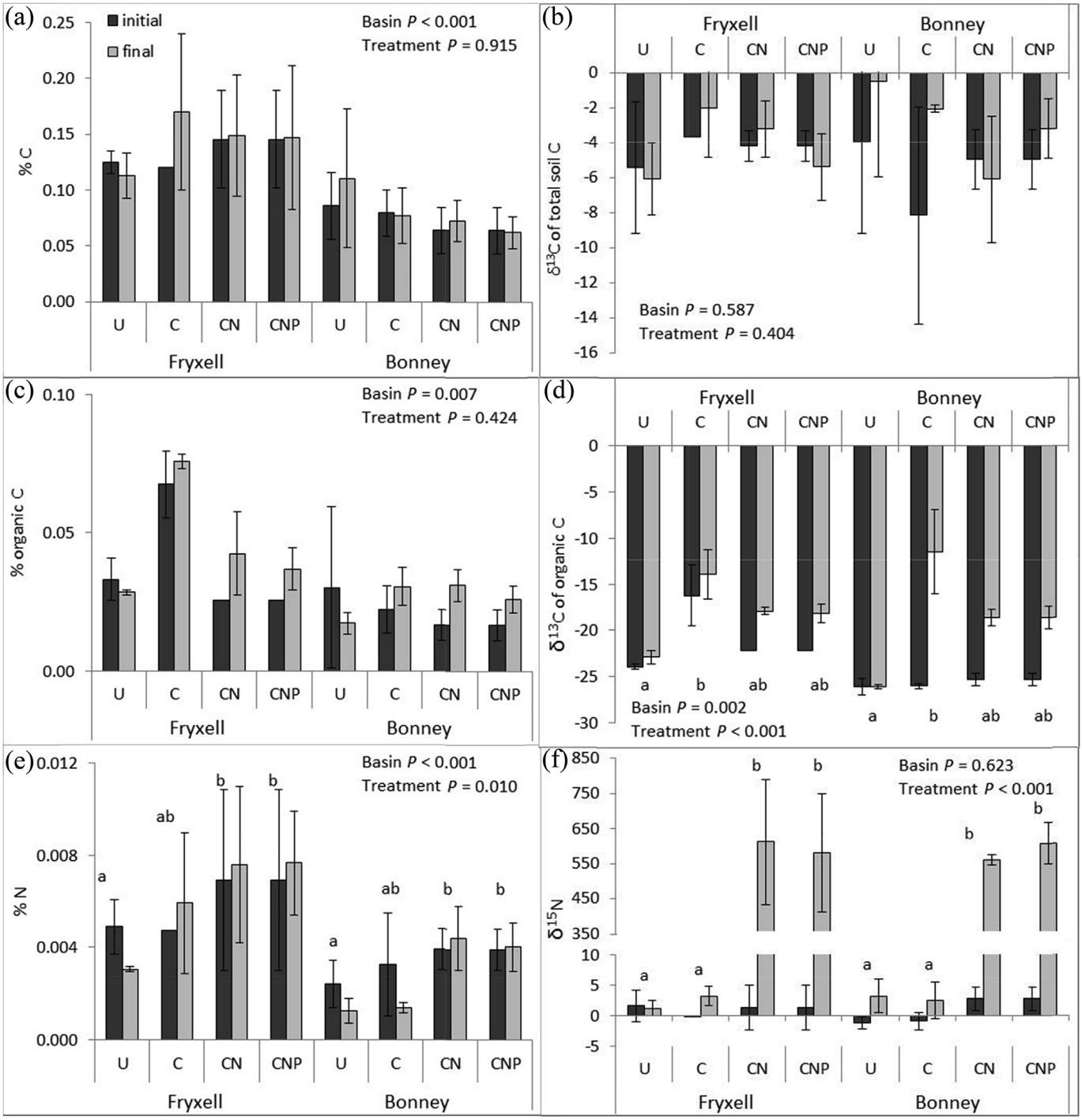

Fig. 5. Content and isotopic composition of soil total C, organic C, and N from two lake basins over the course of a laboratory incubation. Soils from each basin received one of four treatments: untreated (U), ^{13}C addition (C), ^{13}C and ^{15}N addition (CN), and ^{13}C, ^{15}N, and P addition (CNP).

4. Discussion

Elevated soil respiration (relative to W plots) in C and N treatment plots indicates that Taylor Valley soils are co-limited by these two resources. Such limitation has been identified previously for this ecosystem, though some laboratory incubations also show a modest influence of C and N alone that we did not detect in this field manipulation (Hopkins et al., 2006; Dennis et al., 2012; Ball and Virginia, 2014). Our results support our hypothesis that release from C limitation is necessary to observe a response to nutrient additions, as the low C:N ratio of these soils would suggest, but we did not find support for the second hypothesis that native soil stoichiometry influenced these results, given that both basins with their differing P availability and resulting elemental ratios exhibited higher soil respiration under C and N together. Even when P is available outside of stoichiometric ratios necessary for balanced growth, it appears to have little influence on biological activity, except perhaps only for a very short-lived stimulation of respiration and denitrification in the Bonney Basin (where native P is lower) when added with C and N in the laboratory incubation, and a greater abundance of *Eudorylaimus* sp. under P compared to both N treatments (N and CN). Other studies in vastly different ecosystems have also seen a suppression of invertebrates after

N enrichment, as we see in soils from the Lake Bonney basin, attributed to acidification reducing food resources and mineral cation concentration (Chen et al., 2015). However, in our study pH and organic C do not mirror the nematode response to N and CN, and the decrease in *Eudorylaimus* sp. at Bonney under these treatments is likely due to direct toxicity from the N ions (Nkem et al., 2006).

Notably, it took several years before the stimulation effect on CO_2 flux showed a measured response, suggesting the soil community present at the start of the treatments was not initially able to make use of the release from resource limitation. A similar delay was noted in another study from this region (Sparrow et al., 2011). Other studies have suggested a shift in microbial community composition is first required, e.g. a shift between r- and K-strategists, or first requiring an increase in available substrate before the community responds (Rinnan et al., 2007; Heuck et al., 2015). Notably, Hopkins et al. (2008) also observed stimulation of CO_2 flux in soils collected from a nearby dry valley three years after adding a C and N source, with no accompanying change in microbial community structure, suggesting it is possible that the response is due to a gradual shift in physiological capabilities and biomass rather than a shift in taxa. They also note that enzyme activity was stimulated by C + N addition under the stable microbial community composition and suggest that added C may be used preferentially for respiration over biosynthesis processes. Our data support this, given the increase in respiration under the CN treatment without an increase in microbial biomass or invertebrate abundance. A lack of change in microbial biomass in response to C and N additions, despite increased respiration, has been seen in other ecosystems, where microbial stoichiometry rather than biomass dominates the response (Buchkowski et al., 2015).

In both basins, the large majority of soil C is inorganic (approximately 80–90%). Abiotic fluxes of inorganic C as CO_2 can be a measurable portion of overall CO_2 flux at this site, driven by temperature-dependent dissolution of CO_2 in soil water (Ball et al., 2009; Shanhun et al., 2012; Ball and Virginia, 2015). Because all treatments at a given site were measured within a short time frame, under fairly constant temperature and moisture conditions, we anticipate that the differences among treatments, and therefore the pulse of CO_2 under CN treatments, are the result of biological activity. This stimulated activity might be the result of the microbial and/or invertebrate community. Across ecosystems, few studies report invertebrate responses to nutrient fertilization alongside biogeochemical data, as we do here. While invertebrate abundance did not reflect the soil CO_2 flux response to fertilization, it is possible that like the microbial community, their activity can be stimulated without a resulting change in abundance, impacting biogeochemical cycling. Nematode abundance in this ecosystem can take years to increase in response to environmental change, given their slow rate of development to reproduction (Knox et al., 2016), while nematode activity can respond very rapidly (Treonis and Wall, 2005). Further, we see a response to the treatments by currently low-abundance invertebrates such as *Eudorylaimus* and tardigrades, noted in other studies as a response to moisture availability (Andriuzzi et al., 2018), suggesting that long-term pulses that might result from future climate change will begin to impact the biogeochemical processes for which these taxa are responsible as they increase in population.

After the delayed onset of stimulated respiration, the stimulation was noticeable for several years before diminishing again by year 6. Some of this might be due to interannual variability in climate factors that influence CO_2 flux, but soil temperature and moisture are not drastically different between years 3–4 and 5–6. Thus it is possible that the diminishing stimulation of CO_2 flux by year 6 might indicate a loss of the treatment effect, though the reason is uncertain. One possibility is that another resource becomes limiting, such as a micronutrient not measured in our study. Micronutrients such as Mn and K can limit soil communities and C turnover processes in other ecosystems (e.g., Bowker et al., 2005; Stendahl et al., 2017), and it is possible that in these low-organic soils, multiple nutrients can quickly become limiting beyond C, N and P. Another possibility is that the buildup of NO_3^- and NH_4^+ in the CN treatment began inhibiting activity in comparison to previous years, but this seems less likely given that overall conductivity (a correlate for salinity) did not significantly differ among treatments. A final possibility is the length of time between treatment application and measurement of CO_2 flux, given that a longer period of time between application allows time for the treatment effect to diminish. To maintain plot integrity over the long-term, we were not able to sample repeatedly after treatment application to measure the rate at which the treatment effect diminished, and in years 3 and 4 the flux measurements were made within a few days of treatment application, wheras in years 5 and 6, logistic constraints led them to be made 7–12 days after treatment. Thus, it is possible that the later years' measurements were made towards the end of the pulse. In the incubation, the pulse only lasted 23 days, but this was at sustained temperatures on the high end of what *in situ* soils would experience in the dry valleys. While the dark-colored soil in the dry valleys can approach 20 °C on a sunny day, the laboratory incubation sustained these temperatures for a longer duration than the soils would experience naturally. It is possible therefore that the stimulation was shorter-lived in our incubation than it would be in the field, due to the community's lack of adaption to deal with these sustained high temperatures.

Our results suggest that meltwater pulses accompanied by pulses of C and N have the potential to influence dry valley nutrient cycling. Gaseous losses of both C and N were stimulated by resource additions. However, the pulse is relatively short lived, at least under optimal laboratory conditions, so the amount of C and N lost may not significantly influence nutrient cycling over a long-term time scale. Indeed, total %C in soil was not significantly reduced over the 6-year extent of the experiment. The laboratory incubation suggests that addition of the labile C stimulated use of soil C, given that only a portion of the difference in C mineralized under CN and CNP addition can be attributed to the labeled mannitol, this did not result in a significant decrease in total soil C. Such priming has been recorded in other studies (Nottingham et al., 2009; Jenkins et al., 2010), and occasionally also without an impact on extant soil organic C (Ziegler and Billings, 2011). Further, while denitrification was stimulated by N addition in the laboratory incubation, it was only stimulated once anaerobic conditions had been created by flushing with N_2 gas. Denitrification is not considered a significant process in the arid Antarctic soils, where anaerobic conditions are less likely to be sustained except along stream margins or in soils saturated by lake level rise.

Despite both basins being CN co-limited, we observed other differences between the basins, reflecting their different edaphic properties. CO_2 flux overall is higher in Fryxell soils, where *in situ* C and N are higher and microbial and invertebrate abundance are greater. The greater abundance of these limiting resources and a larger community allow for greater biological respiration. The size of the CO_2 pulse resulting from C and N addition is also greater at Fryxell, demonstrating a greater capacity of the Fryxell soil community to make use of the excess resources, whereas communities in Bonney soils might be too strongly limited by other environmental factors (such as temperature or moisture) to make use of them. Additionally, as noted above, Bonney soils do respond slightly to P and CP compared to Fryxell soils (where *in situ* P is naturally higher), suggesting a potential, albeit small, influence of *in situ* stoichiometry on the response to future resource pulses, particularly if those future pulses alleviate the water and CN limitation. Interestingly, while invertebrate abundance is generally higher at Fryxell, *Eudorylaimus* sp. is approximately equivalent in the two basins' unamended (U) treatments, but lower in Fryxell soils under most other treatments due to a decrease in abundance compared to the controls. The reason for a decrease in Fryxell soils but not Bonney is unclear. As algal feeding omnivore-predators, *Eudorylaimus* sp. abundance in these basins is correlated positively with soil moisture and chlorophyll α and negatively with temperature (Barrett et al., 2008b; Simmons et al., 2009; Shaw et al., 2018). However, soil moisture and temperature

cannot explain the different response to treatments in the two basins, given that treatments involved water addition to increase moisture, and soil temperature would not likely increase with water application. A remaining possibility is that algal abundance decreased at Fryxell under treatment application (without decreasing overall % organic C), but this was not measured in our study. Overall, while both basins will respond to future pulses through potential release from CN limitation, the magnitude of their response will likely differ, as a result of their different geologic legacies impacting *in situ* stoichiometry, organic matter, and biological communities.

In conclusion, we identified a co-limitation of C and N on biotic activity in this dry valley ecosystem, regardless of differences in *in situ* C:N:P ratios, not supporting our hypothesis that differences in native biogeochemistry resulting from landscape history would influence the impact of nutrient addition. We have evidence that the fertilization predicted to accompany meltwater pulses with future climate change will impact C and N losses due to microbial activity, but we do not have evidence that it will have a strong influence on soil C and N stocks in this ecosystem unless pulses are sustained long-term.

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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.2018.03.025.

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