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Agricultural Management and Labile Carbon Additions Affect Soil Microbial Community Structure and Interact with Carbon and Nitrogen Cycling

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Abstract

We investigated how conversion from conventional agriculture to organic management affected the structure and biogeochemical function of soil microbial communities. We hypothesized the following. (1) Changing agricultural management practices will alter soil microbial community structure driven by increasing microbial diversity in organic management. (2) Organically managed soil microbial communities will mineralize more N and will also mineralize more N in response to substrate addition than conventionally managed soil communities. (3) Microbial communities under organic management will be more efficient and respire less added C. Soils from organically and conventionally managed agroecosystems were incubated with and without glucose (^{13}C) additions at constant soil moisture. We extracted soil genomic DNA before and after incubation for TRFLP community fingerprinting of soil bacteria and fungi. We measured soil C and N pools before and after incubation, and we tracked total C respired and N mineralized at several points during the incubation. Twenty years of organic management altered soil bacterial and fungal community structure compared to continuous conventional management with the bacterial differences caused primarily by a large increase in diversity. Organically managed soils mineralized twice as much NO_3^- as conventionally managed ones (44 vs. 23 μg N/g soil, respectively) and increased mineralization when labile C was added. There was no difference in respiration, but organically managed soils had larger pools of C suggesting greater efficiency in terms of respiration per unit soil C. These results indicate that the organic management induced a change in community composition resulting in a more diverse community with enhanced activity towards labile substrates and greater capacity to mineralize N.

Introduction

In most unmanaged ecosystems, soil carbon (C) and nitrogen (N) cycles are tightly linked through microbial processes, such as decomposition and N mineralization, but conventional agricultural practices decouple these cycles. Nitrogen fertilizer application in conventional systems aims to replace microbially mediated N mineralization and supply N directly to plants. Though this shift to fertilizer N can increase crop production, fertilizer application requires energy consumption and can have deleterious impacts through increased leaching and gaseous losses of N [1–4]. In contrast, organic management practices, particularly crop rotations including legumes and cover cropping, can produce a more tightly coupled cycle of C and N and reduce N losses [5]. However, how soil microbial communities, agricultural management, and soil processes, such as decomposition and N mineralization, influence each other are not yet well understood [6–8].

The composition and activity of the soil microbial community is influenced by ecosystem state factors (e.g., climate, soil type), plant species composition and, farming practices in agroecosystems [9–11]. Organic management practices using diverse rotations and additions of organic residues affect microbial community structure over the long term through buildup of soil organic matter (SOM) and changes in SOM chemistry [6, 7, 12–14]. In addition, greater crop diversity alters plant litter inputs and can presumably increase the number of ecological niches available for soil microbes [7, 11]. The structure of microbial communities can in turn have important implications for the rates of soil processes. For example, variation in microbial community structure in soils has been observed to influence rates of denitrification, nitrification, and nitrogen fixation [15–20]. This suggests that long-term organic management will shift microbial community composition and diversity relative to conventionally managed soils and that these changes will have qualitative and quantitative impacts on soil processes [7, 9, 21, 22].

Labile substrates, such as root exudates or litter leachates, are the key link between rates of decomposition, N mineralization, and the structure of the microbial community. Since heterotrophic soil microbes are most commonly limited by carbon availability, increases of easily used carbon substrates can lead to rapid increases in microbial biomass and decomposition of more recalcitrant SOM [23–25]. This increased rate of decomposition, also known as the priming effect, can also increase N mineralized from recalcitrant organic matter [26]. Increased N mineralization increases the amount of N available to plants [26, 27]. This coupled cycling is particularly important in minimizing potential leaching losses of inorganic N, since unlike synthetic fertilizer application, this process leads to N being mineralized in response to plant demand and hence more likely to be assimilated by plants and less likely to be lost via leaching and other N loss pathways [12, 26, 28].

This project examined the impact of agricultural management on microbial community structure, and the ability of these communities to use labile carbon substrates and to mineralize N. We examined these questions using microcosms with soil from adjacent fields with identical soil types, but contrasting management regimes. We used multiple small pulsed C substrate additions that mimic plant root inputs to test for interaction between management type and labile substrate availability. We tested several specific hypotheses. (1) Management will lead to distinct soil bacterial and fungal communities with greater diversity in organically managed soils than

conventionally managed systems. (2) Organically managed soils will mineralize more N than conventionally managed soils and will also mineralize more N in response to substrate addition. (3) Microbial communities from organically managed soils with higher quality and quantity of SOM will be more efficient and respire less added substrate than conventionally managed soils. The results of this study will elucidate mechanisms that link soil microbial communities, plant root inputs, and soil N supply.

Methods

Site Description and Soil Sampling

We identified four sites within 3 km of Penn Yan, NY, USA (42°39′N, 77°3′W), located on the same soil series, Honeoye (fine-loamy, mixed, active, mesic Glossic Hapludalfs). Two sites were conventionally managed for field corn and occasionally legumes (Conv sites). The other two sites were organically managed with occasional additions of composted manure and a multiyear rotation of soybean, winter wheat, red clover (plowed down as a green manure), field corn, kidney beans, and spelt (Org sites). All sites were tilled, either in preparation for planting (Conv) or for weed control (Org). The Org sites had been managed organically for 20 years at the time of sampling. Prior to transition to organic practices, the Org sites had been conventionally managed with rotations and practices similar to the Conv fields. Earlier work at these farms indicates that the Conv fields receive greater total N inputs, mainly in the form of fertilizer N, while the Org fields operate with a smaller N surplus derived mostly from legumes [29]. All sampling plots were placed in areas of the fields that were planted with corn the previous planting season and were harvested prior to soil sample collection. Three of the sites were adjacent to each other but separated by drainage ditches with the fourth less than 500 m away separated by a road. We measured several background soil biogeochemical characteristics to establish basic differences between managements (Table 1).

Table 1

Initial soil characteristics

Management	pH	Total C (%)	Total N (%)	TEC	TEN	MBC	MBN	fPOM C	fPOM N	oPOM C	oPOM N
Conventional	6.85 (0.31)	*0.96* *(0.04)*	*0.09* *(0.007)*	21.3 (2.8)	4.38 (1.3)	624 (57)	20.7 (0.10)	586 (45)	26.6 (0.79)	1005 (258)	53.0 (11)
Organic	7.04 (0.25)	*1.22* *(0.05)*	*0.12* *(0.01)*	25.0 (0.85)	8.97 (2.2)	620 (68)	22.3 (0.47)	541 (99)	26.2 (4.3)	1308 (88)	80.7 (6.9)

All analyses in units of μg C or μg N g^{-1} dry soil, except pH and total soil C and N in % by dry soil mass. Values represent means with standard errors in parentheses. Total soil C and total soil N (in *italics*) differed significantly between organic and conventional (ANOVA, $p < 0.05$)

TEC or TEN = total 2 M KCl extractable C or N, MBC microbial biomass C, MBN microbial biomass N, fPOM free particulate organic matter, oPOM occluded particulate organic matter

We sampled the top 10 cm of soil in January 2010 after gently removing the snow pack layer (approximately 20 cm deep at the time of sampling). Samples were collected by driving a 10-cm-diameter stainless steel core into the soil then removing the intact core by excavating gently around the outside perimeter of the core. At each site, we collected three cores in random locations 10 m apart, which were later composited to reduce the effects of fine scale spatial heterogeneity. Samples were stored on ice in polyethylene bags for transport back to the laboratory. To simulate spring thawing, field frozen soils were thawed at 4 °C. The bottom of the core was covered with cheese cloth to allow melt water to drain. Soils were thawed for 2 days and sieved (2 mm) to remove large rocks and roots. Sieved soils were stored at 4 °C for less than 24 h before subsampling. Subsamples were oven dried at 105 °C to determine gravimetric soil moisture, stored at −80 °C for later DNA extraction, or transferred to microcosms.

Incubation Conditions, Isotopic Labeling, Leachate Collection and Analysis of Mineralization

The goal of our microcosm design was to simulate conditions where the soils received periodic rainfall events and bursts of root exudation. To accomplish this, soil microcosms were constructed from disposable liquid filtration units (Fig. S1). This design had the added advantage of allowing us to add substrate in liquid form while controlling soil moisture content and preventing buildup of soluble N molecules, like nitrate (NO_3^-), from microbial processes. Each microcosm had a glass fiber filter (Whatman 934-AH) secured with silicone sealant above a 0.2-μm PES membrane. A 50-g dry equivalent of soil was placed on top of the glass fiber filter around a 2.5-cm-diameter plug of loosely packed PTFE chips to facilitate drainage. For each site, four microcosms were constructed. To measure net N mineralization, 25 ml of 0.01 M calcium chloride ($CaCl_2$) was added gently to the soil surface (and avoiding the PTFE plug) and filtered into a collection vessel using a vacuum pump. This liquid addition simulates the amount and ionic concentration of a spring rainfall event in this region. Resulting filtered liquid was removed from the collection vessel and stored at −40 °C. Microcosms were incubated at 23 °C in the dark. Simulated rainfall and mineralized N collection was repeated at 3, 13, 28, 58, and 97 days after the incubation began. Ammonium (NH_4^+) and NO_3^- in the filtered solution were quantified colorometrically using the NED-sulfanilamide and hypochlorite–phenolate methods on an ACQ2 autoanalyzer (Seal Analytical, Mequon, WI). Total dissolved organic C was quantified in leachates by Pt-catalyzed combustion in a total organic C analyzer (TOC-V; Shimadzu Corp., Columbia, MD).

For each site (2 Org, 2 Conv), two microcosms were designated "pulsed priming", and two "control". Pulsed samples received 125 μg of 98 % isotopically enriched ^{13}C glucose (50 μg C, or 1 μg C g dry soil^{-1}) after each rainfall and mineralization interval throughout the incubation and were moistened to 50 % water holding capacity with deionized water. The amount of glucose added was chosen based on a goal of ~1 % addition of C of the total microbial biomass C pool. This relatively small priming addition was selected to avoid a possible artifact of "apparent priming", which results from a large dose causing a major growth of microbial biomass followed by a population crash that artificially inflates estimates of respiration derived from SOM priming [23]. The dose also mimics an amount of root exudate that would prime microbial activity in the field. Control microcosms received only deionized water. Water holding capacity of 50 % was

chosen to achieve ~70 % water filled pore space in these soils based on soil texture, which is the optimal water content for respiration [30, 31]. At the conclusion of the incubation (day 97), subsamples of soil were taken for measurement of microbial biomass C and N and genomic DNA (stored at −80 °C). A schematic of the incubation and soil processing is presented in Fig. S2.

DNA Extraction, Amplification, TRFLP, and Quantitative PCR

Genomic DNA from subsamples of soil was extracted using the Powersoil DNA extraction kit (MoBio Labs, Carlsbad, CA). Three replicates of 0.5 g soil per microcosm were extracted to reduce potential bias from soil microsite heterogeneity. DNA was extracted at the beginning and end of the incubation due to the destructive nature of the soil sampling needed to extract DNA. To further purify and concentrate the extractions, the three replicate extractions per microcosm were combined and precipitated with ethanol [32].

We analyzed the microbial community structure using terminal restriction fragment length polymorphism analysis (TRFLP) [33–35]. To perform TRFLP, bacterial 16S rRNA genes were amplified using the universal primers Bac8f with a 5′ end 6-FAM label (5′-AGAGTTTGATCCTGGCTCAG-3′) and unlabelled primer Univ1492r (5′-GGTTACCTTGTTACGACTT-3′) [36]. Fungal 28S rRNA genes were amplified using the fungal universal primers LR0R (5′-ACCCGCTGAACTTAAGC-3′) and LR5 (5′-TCCTGAGGGAAACTTCG-3′) [37, 38]. Each 25 μl PCR reaction contained a final concentration of 1.5 μM of 6-FAM-labeled forward primer, 0.5 μM unlabelled reverse primer, 1× amplitaq gold buffer (Applied Biosystems, Carlsbad, CA), 1 mM magnesium chloride ($MgCl_2$), 0.5 mg ml^{-1} bovine serum albumin (BSA), and ~15 ng template DNA. Cycling conditions were 10 min at 95 °C, 35 cycles of 30 s at 95 °C, 30 s at 50 °C, 45 s at 72 °C, and a final extension of 10 min at 72 °C. We ran three replicate amplifications per sample and composited the products and cleaned them using Wizard PCR cleanup kits (Promega, Madison, WI). Amplifications were replicated to reduce potential PCR biases and to produce enough DNA for subsequent restriction digestion and fragment analysis. Cleaned PCR products were digested with the restriction enzyme *Hae*III (Promega) as per the manufacturer's directions in a 20-μl reaction. The digests were incubated in a 37 °C water bath for 4 h and then incubated in a heating block at 70 °C for 10 min to deactivate the restriction enzyme. Digested PCR products were cleaned with a Performa DTR 96-well cleanup system (Edge Biosystems, Gaithersburg, MD). Cleaned digests were dried in a rotary evaporator and resuspended in 9.85 μl of formamide and 0.15 μl of 500 LIZ size standard (Applied Biosystems). Terminal restriction fragments (TRFs) in the samples were quantified using a 3730XL gas capillary DNA sequencer (Applied Biosystems).

We quantified the abundance of bacterial and fungal small subunit rRNA (16S or 18S) copies using published methods [39, 40]. The primer pair F515 (5′-GTGCCAGCMGCCGCGGTAA-3′) and R806 (5′-GGACTACHVGGGTWTCTAAT-3′) was used for bacteria, and Nu-SSU-0817 F (5′-TTAGCATGGAATAATRRAATAGGA-3′) and Nu-SSU-1196R (5′-TCTGGACCTGGTGAGTTTCC-3′) was used for fungi [39, 41]. To estimate the abundances of rRNA gene copies, we used standard curves from 10-fold serial dilutions of 16S or 18S amplicons generated using the same primers from isolates of *Klebsiella pneumonia* or *Fusarium solani*. Twenty-five microliter reactions were run with 12.5 μl of QuantiFast SYBR Green qPCR

mastermix (Qiagen, Valencia, CA, USA), 0.5 μM final concentration of the forward and reverse primers each, 5 ng of template DNA, and sterile water on a Bio-Rad C1000/CFX96 thermocycler (Bio-Rad, Hercules, CA, USA). We used the manufacturer's suggested 2-step thermocycler conditions of 95 °C for 5 min followed by 40 cycles of 95 °C for 10 s and 55 °C for 10 s.

TRFLP Data Analysis

Size standards were applied to the fragments using Peak Scanner ver. 1.0 (Applied Biosystems). We eliminated all peaks outside the range of the standards (50–800 bp) and any peaks below 50 units of height, and we used the Local Southern method of size calling. Using the online software, T-REX [42], we filtered noisy peaks using a standard deviation multiplier of 1 [43]. We aligned peaks across samples using the peak aligning algorithm in T-REX with a clustering threshold of 0.5 bp [44]. We then relativized peak areas within a given sample to the total peak area within each sample to create a matrix of TRF relative abundance by sample and averaged the two laboratory replicate microcosms per treatment [44]. To normalize the data, we took the square root of the relative abundance for subsequent analyses. We relativized, averaged the technical replicates, and normalized in order to make all samples directly comparable (i.e., removed background variability due to variation in total sample fluorescence or sequencer read variation), and we used the calculated relative abundance as the input for all subsequent analyses.

Non-metric multidimensional scaling (NMS) was performed in PC-ORD ver. 5.31 (MjM Software, Gleneden Beach, OR, USA) to visualize overall differences in bacterial and fungal TRF patterns across managements and treatments. NMS was performed using the Sorenson/Bray–Curtis distance metric and random starting configurations with 40 runs with real data and 50 runs with randomized data for a Monte Carlo analysis. To test if management type or C treatment had a significant impact on TRF profile, we used Permutation-based Multivariate Analysis of Variance (PerMANOVA) in PC-ORD using the Sorenson/Bray–Curtis distance metric [45]. To identify which TRFs were driving differences between different treatment and management communities, we performed two-way cluster analysis in PC-ORD [45] based on a similar analysis approached used by Angel et al. [46]. Briefly, two-way clustering performs simultaneous hierarchical clustering on both samples (treatments and managements) and TRFs, and produces a two dimensional heat map based on relative TRF abundance with dendrograms for both samples and TRFs. Samples that cluster together in the sample dendrogram have similar compositions of TRFs, whereas TRFs in the TRF dendrogram cluster together when they appear in similar abundance in the same samples. For the clustering analysis parameters, we used the Sorenson/Bray–Curtis distance metric with flexible beta linkage (β = −0.25). Diversity of the microbial community was measured by Shannon's index (H'), which we calculated using EstimateS (Robert K Colwell software, Stoors, CT). The relationship between TRF diversity and true microbial diversity can break down with highly diverse microbial communities, however, with less diverse microbial communities such as those found in agricultural mineral soils H' is a reasonable estimate of true microbial diversity [36, 47, 48], and recent studies have found similar patterns of diversity between soil microbial diversity measured with TRFLP and pyrosequencing [36, 49].

Respiration, Soil C and N, and Microbial Biomass Measurements

To measure total C respired and its proportion of ^{13}C, we placed a CO_2 trap of 25 ml of 0.5 M potassium hydroxide (KOH) in a flask on top of the PTFE plug and sealed the incubator with a plastic lid (Fig. S1). After each simulated rainfall, we removed, saved, and replaced the 0.5 M KOH and resealed the incubators with parafilm. Preliminary studies were conducted to determine the CO_2 saturation point of the traps, and 0.5 M KOH was chosen since it was the lowest concentration that would not saturate over the course of an incubation step. In order to ensure that we had complete and accurate recovery of CO_2, we tested the KOH trap method by placing a KOH trap in an incubator with no soil but 2 ml of 2 M K_2CO_3in a beaker. We added 12 M HCl to the K_2CO_3 using a syringe through the parafilm seal to release 2 mmol of CO_2, and we found we could measure CO_2 produced after 24 h within 2 %. We measured the total amount of CO_2 respired by the soils and captured by the KOH by the difference in electrical conductivity of the trap before and after the incubation step [50].We then added strontium chloride ($SrCl_2$) solution in excess (5 ml of 5 M) to the spent KOH trap to form a strontium carbonate ($SrCO_3$) precipitate. Dried ground precipitate was mixed with five times the precipitate mass of vanadium oxide (V_2O_5), and packed it in tin capsules for ^{13}C isotopic analysis. The elemental ratio of ^{13}C and ^{12}C was determined on a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK).

For microbial biomass C and N pools, we used a chloroform fumigation direct extraction procedure both pre and post incubation [51, 52]. Briefly, two 10-g subsamples of soil were placed in 50 ml centrifuge tubes. To one unfumigated tube we added 30 ml of 1 M potassium chloride (KCl), shook longitudinally for 30 min at 120 oscillations/min, and then centrifuged at $10,000 \times g$ for 5 min. We filtered the resulting supernatant through Whatman #1 filter paper and stored at -20 °C. For the other subsample, we suspended a cotton ball above the soil and added 2 ml of chloroform and sealed the tube. The tube was incubated in the dark for 5 days, after which we removed the cotton ball, allowed the tubes to ventilate for 1 h without a cap, and then extracted the soils in the same manner as the unfumigated subsamples. Total amounts of C and N in microbial biomass were determined as the difference between the total C and N in the supernatants of unfumigated and fumigated soil samples. Total dissolved C and N were determined by combustion in a total organic C and N analyzer (TOC-V; Shimadzu Scientific, Columbia, MD, USA). Total KCl extractable C and N were calculated as the amount of C and N present in the unfumigated samples alone. The amount of ^{13}C present in microbial biomass was determined by persulfate oxidation of the 1 M KCl extracts (diluted 20× with distilled water) using an Aurora 1030 W TOC analyzer (OI Analytical, College Station, TX, USA) and subsequent analysis on a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). C-13 analysis was carried out at the UC, Davis stable isotope facility.

Total soil C and N were determined on oven dried and ground samples on a Leco CN-2000 analyzer (Leco Instruments, Lansing, Michigan, US). Particulate organic matter greater than 53 µm (POM) was separated from total SOM using a density and size fractionation method [53, 54]. Briefly, 40 g of dry soil was shaken in a solution of sodium polytungstate (adjusted to a density of 1.7 g cm^{-3}). Lighter free organic material (fPOM) was decanted and filtered through a 53-µm mesh; heavier occluded organic material (oPOM) was shaken with sodium hexametaphosphate to break up aggregates and is then filtered and washed with deionized water through a 53-µm mesh.

Dried and ground POM samples were analyzed for total C and N on a Leco CN-2000 analyzer as above.

Data Analysis for Respiration, N Mineralization, Soil C and N Pools, Diversity, and qPCR

Means and standard errors were calculated for all pool values, and throughout this manuscript we present mean values with standard error of the mean. We used two-way ANOVA by least squares to test for significant differences in pools of C and N, microbial diversity, SSU rRNA gene abundance, and isotopic recovery with a factorial model (including management by C addition interaction) to test for differences between levels of management and carbon treatment using PROC GLM in SAS (SAS institute, Cary, NC, USA).Total C respired, NO_3^-, and NH_4^+ were analyzed using repeated measures ANOVA using PROC GLM in SAS with a factorial combination of management and substrate treatment as main effects (SAS institute).

Results

Initial Soil C and N Pools

Soils from the organically (Org) and conventionally (Conv) managed fields differed initially in both soil organic C and N pools. On average, Org soils had greater amounts of total soil carbon than Conv soils (Table 1). However, soil management did not lead to different amounts of microbial biomass C, KCl extractable C, fPOM C, or oPOM C (Table 1). Prior to incubation, Org soils had greater amounts of total soil N than Conv soils (Table 1). However, other initial preincubation pools of N (microbial biomass N, KCl extractable N, KCl extractable NO_3^- and NH_4^+, fPOM N, and oPOM N) were not significantly different between management types (Table 1). Though there were no differences in the C/N of bulk soil or free POM, occluded POM C/N was 15 % lower in Org than Conv soils (16.3 ± 0.4 vs. 19.2 ± 0.5; $p = 0.04$).

Microbial Community Structure and Abundance

NMS analysis revealed that management type led to differences in bacterial community composition (Fig. 1a; PerMANOVA, $p < 0.05$), but C substrate addition did not significantly alter community structure. Bacterial communities responded to pulsed C addition by becoming more similar along axis 2, although this trend was not significant (Fig. 1b). Management type led to significant differences between bacterial communities both with and without preincubation (T_0) samples included in the analysis (Fig. 1a, b; PerMANOVA, $p < 0.05$). Preincubation bacterial communities were significantly distinct from incubated samples (pulsed and control) within both Org and Conv managements (Fig. 1a; PerMANOVA, $p < 0.05$).
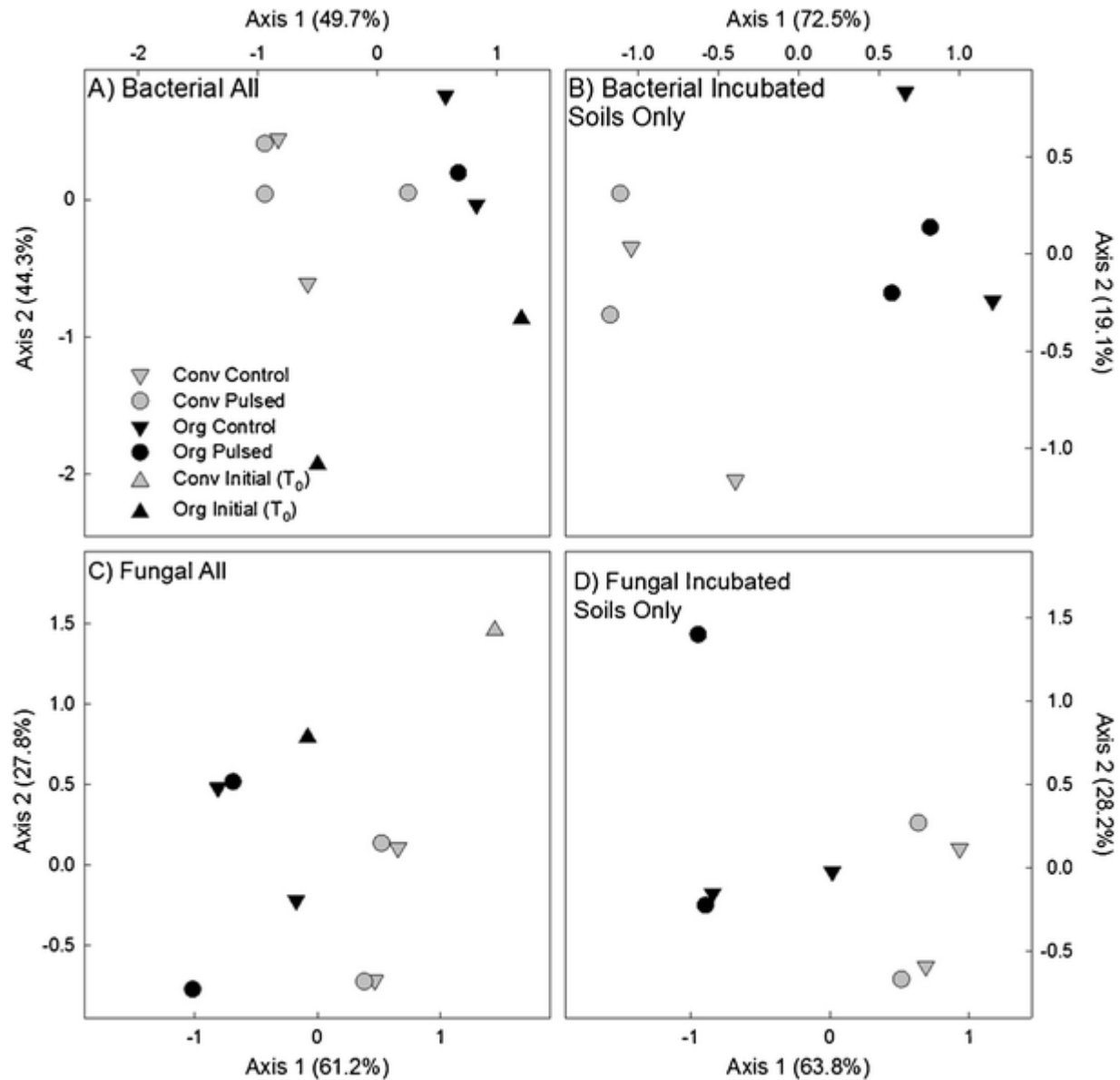
Bacterial and fungal NMS ordination plots. The *x*- and *y*-axes represent the two axes that best summarize the community composition based on TRF relative abundance, and numbers in parentheses by the axes are the amount variation in community structure represented by the respective axis. a, b patterns of bacterial community TRFs and c, d patterns of fungal community TRFs. b, d Bacterial and fungal NMS run without the T_0 sample in the analysis. In all panels, management significantly differentiated communities (PerMANOVA, $p < 0.05$), but carbon treatment did not. a, c T_0 samples were also different from incubated samples within managements (PerMANOVA $p < 0.05$)

Similar to bacteria, fungal communities also differed between Org and Conv soils (Fig. 1c; PerMANOVA, $p < 0.05$) but did not differ between pulsed C substrate addition and control. In addition to management type, fungi differed significantly by plot location suggesting greater spatial differences between individual fields than bacterial communities (Fig. 1c, d; PerMANOVA, $p < 0.05$). Management was also a significant driver of fungal community differences both with and without preincubation samples included in the analysis (Fig. 1c, d; PerMANOVA, $p < 0.05$).

Diversity of microbial communities differed with respect to management history, but not between pulsed C addition and control. In post incubation soils, Org soils had a higher bacterial TRF diversity based on relative abundance (H′ = 3.43 ± 0.012) than Conv soils (H′ = 3.32 ± 0.042, p = 0.02). Fungal communities did not differ in diversity between Conv (H′ = 3.64 ± 0.108) and Org soils (H′ = 3.74 ± 0.013). Neither bacterial nor fungal communities differed in diversity between pulsed C addition treatments when controlling for management types.

Similar to NMS bacterial communities clustered by management (Fig. 2, y-axis dendrogram); however, two-way clustering identified 17 out of 55 bacterial TRFs (31 % of all TRFs) that were in high abundance only in the Org soils (Fig. 2, x-axis dendrogram). Another set of bacterial TRFs (24 of 66, 44 %) were ubiquitous and abundant across all samples. The remaining bacterial TRFs were less common and distributed across Org samples.
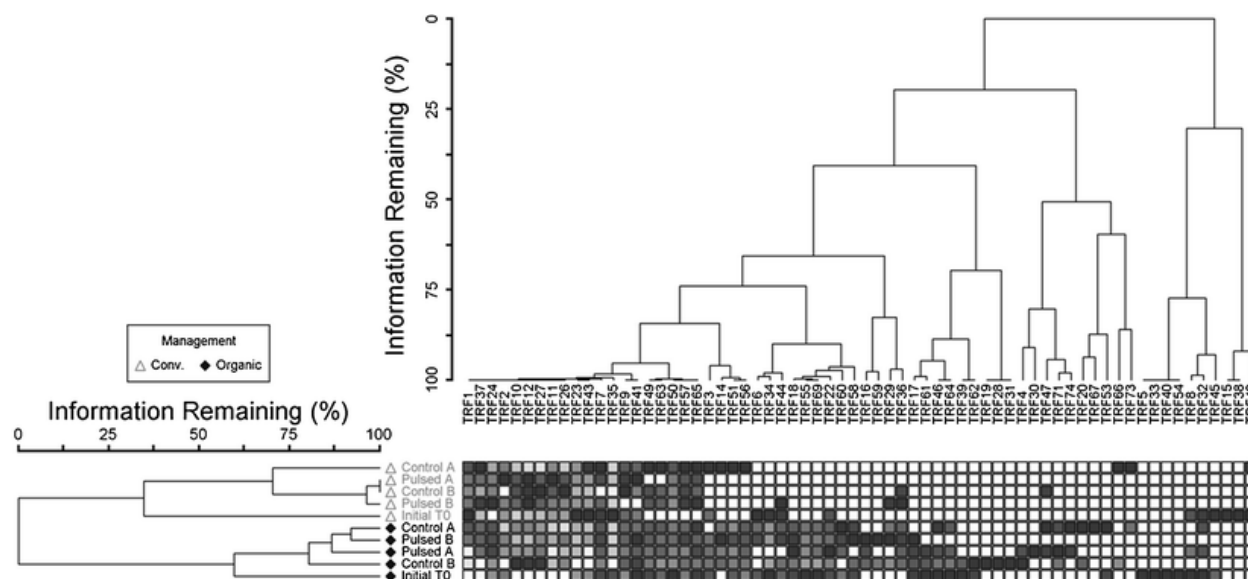

Fig. 2

Two way cluster analysis of bacterial community TRF relative abundance. *Shading* of cells indicates relative abundance of each TRF in each plot, management, and treatment with *white* indicating absence and *darker colors* indicating increasing relative abundance. TRFs that cluster closely on the *x*-axis dendrogram appear in similar abundance in similar samples, and samples that cluster closely on the *y*-axis dendrogram have similar compositions and abundances of TRFs

Two-way cluster analysis also confirmed the NMS and PerMANOVA results that fungal communities clustered strongly by management type and by plot (Fig. 3, *y*-axis dendrogram). However, unlike bacterial communities, fungal TRF distribution did not reveal any distinct TRF groups that corresponded with particular managements or substrate addition treatments (Fig. 3, *x*-axis dendrogram). Nearly half of the fungal TRFs (65/141) were abundant and ubiquitous across all managements and treatments (Fig. 3, *x*-axis dendrogram). The remainder showed patchy distribution with high abundance in individual fields suggesting that a large number of fungal taxa were unique to specific plots/fields irrespective of management type or carbon treatment.
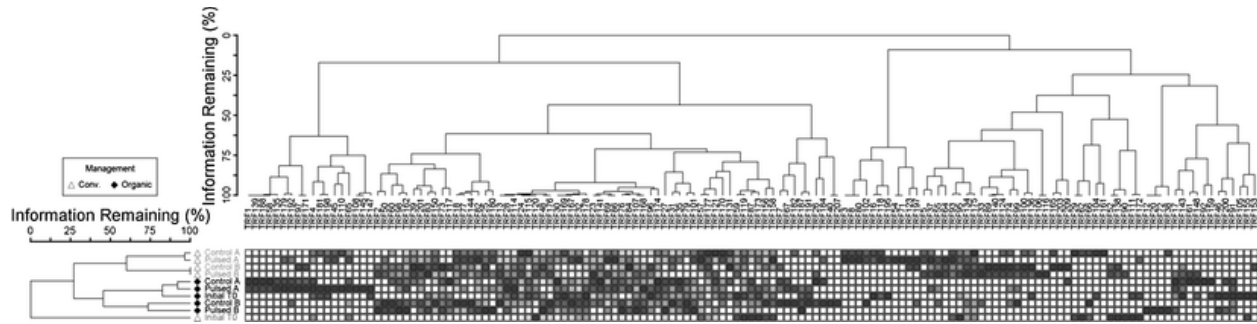
Fig. 3

Two-way cluster analysis of fungal community TRFs. Shading of cells indicates relative abundance of each TRF in each plot, management, and treatment with white indicating absence and darker colors indicating increasing relative abundance. TRFs that cluster closely on the x-axis dendrogram appear in similar abundance in similar samples, and samples that cluster closely on the y-axis dendrogram have similar compositions and abundances of TRFs

Similar to our chloroform fumigation–extraction biomass measurements, neither bacterial 16S nor fungal 18S rRNA gene copy abundance differed between management histories or C substrate treatments. However, preincubation soils had more bacterial 16S rRNA gene copies ($1.3 \times 10^9 \pm 0.2 \times 10^9$ copies g dry soil^{-1}) than post incubation soils ($0.48 \times 10^9 \pm 0.07 \times 10^9$ copies g dry soil^{-1}), and this difference was significant (ANOVA, $p < 0.05$). Fungal 18S rRNA gene copy abundance did not differ significantly between preincubation soils ($20 \times 10^6 \pm 5.5 \times 10^6$ copies g dry soil^{-1}) and post incubation ($10 \times 10^6 \pm 3.9 \times 10^6$ copies g dry soil^{-1}).

Respiration and N Mineralization During Incubation

We found no significant treatment effects on the processing of newly added ^{13}C substrate and no significant effects on ^{13}C fluxes (Table 3). There was no effect of management or substrate treatment on total C respired over the course of the incubation (Fig. 4). There was also no effect of substrate treatment or management type on the amount of dissolved C filtered from the soils in simulated rainfall events; values ranged from 11.1 ± 0.24 μg C flushed g soil^{-1} to 12.4 ± 0.51 μg C flushed g soil^{-1}. There was no difference in total respired ^{13}C substrate between soil management types.
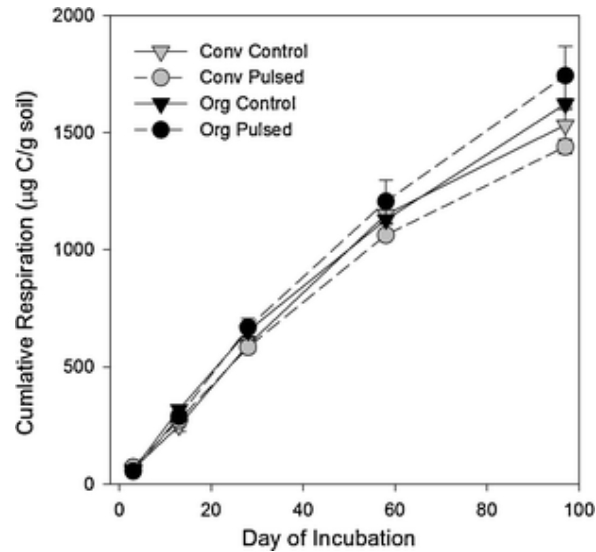
11

Fig. 4

Cumulative soil respiration during incubation. *Black symbols* indicate organic and *grey symbols* conventional management; *triangles* are deionized water controls, *circles* are pulsed ^{13}C glucose incubations

Though management and substrate treatment did not have significant effects on total C respired, Org soils had twice as much N mineralized (virtually all NH_4^+ was nitrified) during the incubation as Conv (46 vs. 24 µg N g soil^{-1}; Fig. 5, $p < 0.001$). In addition, over the course of the incubation C addition stimulated NO_3^- production in Org relative to control (52 vs. 39 µg N g soil^{-1}, $p = 0.03$), whereas C addition had no impact on NO_3^- production in Conv soils (Fig. 5). The effect of substrate addition on mineralization in Org became more dramatic over the course of the incubation (Fig. 5). The amount of NH_4^+ produced by the soils during the incubation was several orders of magnitude lower than NO_3^- without any significant differences between managements or carbon treatments (data not shown).
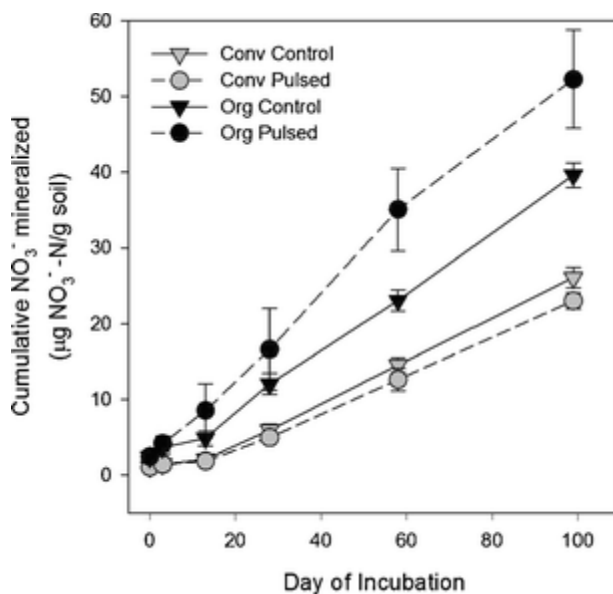
Fig. 5

Cumulative soil mineralization (only nitrate detectable). *Black symbols* indicate organic and *grey symbols* conventional management; *triangles* are deionized water controls, *circles* are pulsed ^{13}C glucose incubations. Organically managed soils produced more NO_3^- than conventional ($p < 0.05$), and organically managed soils with pulsed substrate addition mineralized more N than controls ($p < 0.05$)

Post Incubation C and N Pools

Post incubation, microbial biomass N did not differ between management histories or carbon addition treatments, though it was lower across all post incubation treatments compared to preincubation levels (Table 2). Following incubation, KCl extractable N was 34 % higher in organically managed soils than conventional soils. KCl extractable N in organically managed soils was also significantly higher with pulsed carbon addition relative to control (32 %, Table 2). These results were similar to those for KCl extractable NO_3^-; NO_3^- levels did not differ in conventionally managed soils as a function of carbon treatment, but NO_3^- pools were 73 % greater in Org that received pulsed C addition relative to controls that did not receive C amendment (Table 2). In contrast, KCl extractable NH_4^+ concentrations were at least an order of magnitude lower in all samples than those of NO_3^- and were highly variable (Table 2).

Table 2

Total soil C and N pools, post incubation

	Total Soil C (%)	Total Soil N (%)	TEC	TEN	MBC	MBN	KCl NH3	KCl NO3−
Control								
Conventional	0.95 (0.03)	0.09 (0.003)	19.6 (0.67)	11.5 (1.8)	445 (24)	10.5 (2.1)	0.75 (0.35)	2.4 (0.13)

	Total Soil C (%)	Total Soil N (%)	TEC	TEN	MBC	MBN	KCl NH$_3$	KCl NO$_3^-$
Organic	1.20 (0.04)	0.12 (0.006)	29.9 (5.9)	17.8 (0.99)	488 (29)	12.8 (0.40)	0.90 (0.22)	3.9 (0.38)
Pulsed C addition								
Conventional	0.82 (0.08)	0.08 (0.009)	19.9 (1.7)	12.6 (1.7)	487 (27)	9.7 (2.4)	1.2 (0.26)	2.5 (0.16)
Organic	1.25 (0.04)	0.12 (0.010)	31.7 (2.1)	23.6 (3.0)	559 (56)	12.0 (2.3)	0.31 (0.18)	6.8 (1.1)

All analyses in units of μg C or μg N g^{-1} dry soil, except total soil C and N in % by dry soil mass. Values are means with standard errors in parentheses. Total soil C and N, TEC, TEN, and NO$_3^-$ differed between conventional and organic management (in italics, $p < 0.05$), and NO$_3^-$ also differed between control and pulsed C ($p < 0.05$). There were no significant interactions between management and C addition treatments for any variable.

TEC or TEN = total 2 M KCl extractable C or N, MBC microbial biomass C, MBN microbial biomass N

Soil C data indicated that there were shifts in pool sizes during the incubation, but these changes were unrelated to management or C substrate treatment. Neither total soil C nor microbial biomass C were affected by glucose addition or management (Table 2), but post incubation, Org soils had nearly double the KCl extractable C of conventional soils (Table 2, $p = 0.005$). Microbial biomass C decreased significantly during the course of incubation. At the end of incubation the majority of the ^{13}C substrate was retained in the bulk soil (between 34 % and 45 %, Table 3).

Table 3

C-13 budget

	^{13}C added (μg ^{13}C/g soil)	%^{13}C Respired	%^{13}C MBC	%^{13}C Total Soil
Conventional	5	28.4 (2.0)	10.6 (2.1)	41.5 (1.7)
Organic	5	27.3 (2.7)	7.4 (.97)	44.8 (4.4)

Total 2 M KCl extractable C was measured, but all values were below detection limit

MBC microbial biomass C

Discussion

The goal of this project was to examine three hypotheses about the impact of agricultural management on soil microbial community composition and its interactions with C substrates and N mineralization. In agreement with our hypothesis, management produced distinct soil bacterial and fungal communities, and organically managed soils were more diverse in bacterial TRFs. Fungal communities did not differ between management types due to greater spatial variability. Also in agreement with our hypotheses, organically managed soil microbes mineralized more N than conventionally managed soil microbes, and N mineralization in organically managed soils

increased in response to amendment with labile C. Although we expected less respiration from organically managed soils there was no difference between managements, but organically managed soils had larger C pools indicating more efficient respiration per unit soil C.

Our finding that management history had a stronger impact than substrate addition on microbial community diversity and structure is consistent with other studies, which found that management had a long lasting legacy effect on microbial community structure [8, 11, 13, 14]. Bacteria in organically managed soils had a large number of TRFs (one-third of all TRFs) that were unique to that soil community, while all TRFs present in conventionally managed soils were shared with organically managed soils (Fig. 2). The increased diversity of plant inputs and its impacts on SOM could be expected to increase niche diversity in organically managed soils, and this provides one potential explanation of the difference in bacterial diversity between organic and conventional soils [10, 21, 55]. An alternate explanation for the decreased diversity of the microbial community in conventionally managed soils is that higher inputs of inorganic N in the form of fertilizer led to enrichment of microbial groups that were well suited to high mineral N environments at the expense of other groups. This process has been observed in forest soils [56, 57] and could be acting in concert with increased niche diversity in organically managed soil microbial communities to drive diversity differences. The variability of fungi between sample sites within management types (Fig. 1) agrees with other observations of uneven spatial distribution of soil fungi [58, 59].

Interestingly, we observed greater N mineralization in organically managed soils but no difference in microbial biomass (Fig. 5, Tables 1 and 2). Organic management practices have been shown previously to increase the rate of soil processes without causing increases in microbial biomass [8, 10, 21, 24, 55, 60]. Other studies have also similarly shown that when exposed to small additions of carbon substrate the rate of microbial processes can increase without a change in standing biomass [24, 55]. These observations are consistent with the model proposed for the soil microbial loop, in which microbial growth is matched by increased predation allowing rates of respiration and mineralization to increase without changes in the standing stock of microbial biomass [61–66]. Another potential explanation for increased N mineralization in organically managed soils is a change in the community composition of nitrifying organisms, since different groups of nitrifying bacteria display different nitrification kinetics [19, 20, 67–69].

Despite increased rates of N mineralization, we observed that organically managed soils still accumulated N and had a lower C/N, and these differences were most striking in the particulate OM pools (Table 1) [8, 28, 29, 70]. The accumulation of N under organic management has been linked to higher quality (lower C/N) and greater diversity of plant inputs. These differences then influence soil microbial communities and their ability to retain N [70–72].

The muted response of soil respiration to substrate addition (Fig. 5) was contrary to our predictions but not unreasonable since management differences have been accumulating differences in SOM and microbial communities for nearly 20 years, whereas our C additions were small and applied on much shorter time scale [8, 10]. Our carbon additions were deliberately small to better mimic root exudation rates and to avoid potential priming artifacts [23, 25, 73]. Small additions of substrate typically alter some process rates, but much larger additions would be required to shift microbial respiration against the background of long-term management differences [8, 59, 60, 74].

In addition to long-term management differences between the soils, it is also possible that net total C respired in organically managed soils could have been reduced by increased nitrification both in control and with substrate added (Fig. 5). The higher amount of available SOM suggests that organically managed soils should have respired more C, especially with labile substrate added, but the increased nitrification by autotrophic nitrifiers could have decreased CO_2 evolved from the soil surface (Fig. 5). Likely the thawing of frozen soils, which was designed to mimic the spring thawing which occurs in this climatic zone, led to the spike in microbial biomass C and bacterial 16S rRNA gene copy number observed in the initial samples (Tables 1 and 2). The flush of organic material and increased biomass at soil thawing may have contributed to the muted response of soil respiration to substrate addition, i.e., the increased availability of substrate post thaw and the subsequent pool of dead microbial biomass likely overwhelmed the respiration signal of the smaller C addition.

Despite the muted responses of respiration and the influence of thawing treatment, the substrate treatment and management differences in respired C and mineralized N suggest an interesting difference in the stoichiometric limitation of the total microbial community. Conventional soils respired a similar amount of C in total, but mineralized comparatively little N, which contrasts with organically managed soils that respired a similar amount with much more N mineralized (Figs. 4 and 5). This difference suggests the conventionally managed soil microbial community was more limited by N supply (compared to C), whereas the organic community was more N rich in both bulk and extractable N (Tables 1 and 2). These differences in N availability are further supported by the response to labile C addition. The response of conventional was small, but the N rich organic community accelerated N mineralization in response to C addition. This indicates that organically managed microbial communities had more tightly coupled connections between C availability and N mineralization. This tight connection between labile C and N mineralization in organically managed fields suggests that inorganic N supply in organically managed fields would be elevated in the rhizosphere (with high labile C availability). Increased N availability in the rhizosphere via this process might not be visible in situ due to rapid plant N uptake.

In conclusion, our results suggest that organic management altered the diversity of the soil microbial community, and that these microbial differences led to new dynamics between the cycles of C and N. The microbial differences led to greater potential for N mineralization in response to short term C inputs but with long-term accumulation of N in the SOM of organically managed fields. These responses indicate adjustments to long-term agricultural management practices can take soil microbes and their associated biogeochemical processes into account, and these adjustment can lead to a microbial community that is more able to respond to plant root inputs of labile C and supply endogenous mineral N. Increased reliance on coupled C and N cycles could lead to agroecosystems more capable of relying on endogenous N supply with less need for fertilizer application.

Acknowledgements

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