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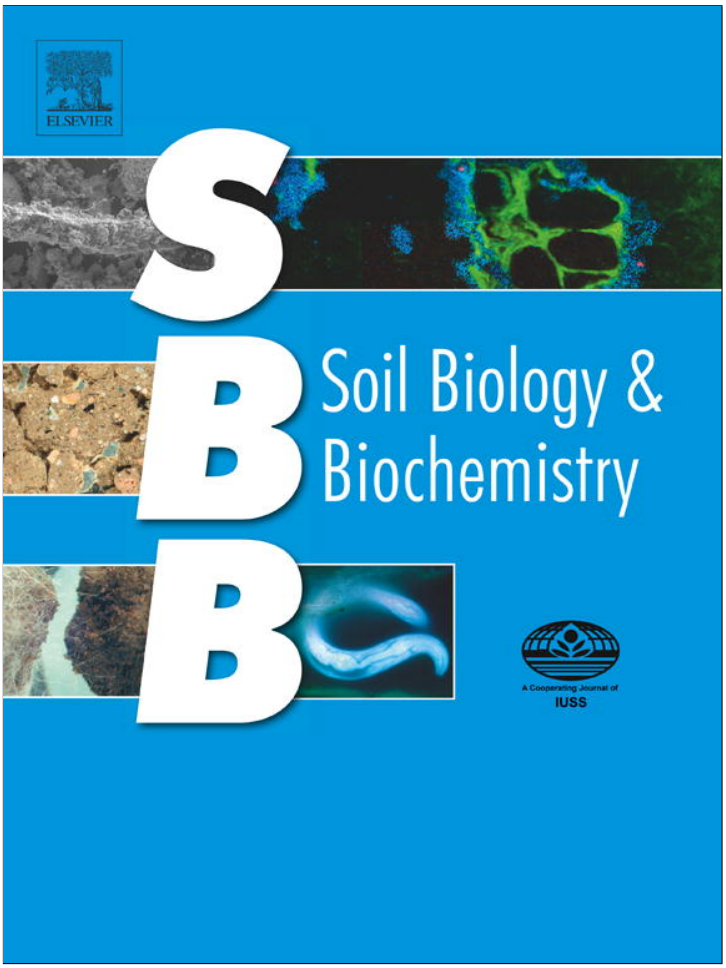

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Evaluation of mechanisms controlling the priming of soil carbon along a substrate age gradient

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ABSTRACT

Soil organic matter (SOM) decomposition has the potential to radically affect carbon dioxide concentrations in the atmosphere. Priming, the increased decomposition of SOM after the addition of a labile carbon (C) source, may be an important regulator of SOM dynamics, yet little is known about the mechanisms of the priming effect. Two hypotheses generated in the last decade have suggested that priming is caused by either the nutrient conditions in soil or the response of the microbial community to labile C addition. We used a three million year substrate age gradient, with associated changes in nutrient availability and microbial communities, to test these two hypotheses. We added ^{13}C labeled glucose to soil in quantities similar to increases in root exudation that can be expected in a high carbon dioxide environment, and traced the effect of C addition on soil C, the microbial community, and soil nutrient pools and fluxes. We observed positive priming, negative priming, and no net priming depending on substrate age. Priming was most positive at the youngest sites with the smallest nitrogen (N) pools, and most negative at the site with the most available N. In contrast, we found no significant relationships between priming and phosphorus availability. Though components of the microbial community size and structure (measured by phospholipid fatty acid analysis) changed as a result of C addition, soil N availability was a better explanatory mechanism of priming effects than microbial community dynamics. Our results suggest close linkages between C and N cycles regulate the magnitude and direction of priming. If general, the stability of SOM in temperate ecosystems is likely to be governed by soil N status.
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1. Introduction

Priming is the phenomenon by which soil organic matter (SOM) decomposes more rapidly after the addition of labile carbon (C) than unamended SOM (Bingeman et al., 1953), and was observed as early as 1926 (Kuzyakov et al., 2000). However, priming is not measured universally: 'negative priming' or 'preferential substrate utilization' are terms that describe less SOM decomposition after C addition, and refer to the utilization of the added C substrate rather than SOM. After decades of research and extensive reviews on the subject (e.g., Kuzyakov et al., 2000; Kuzyakov, 2002; Fontaine et al., 2003; Blagodatskaya and Kuzyakov, 2008), we are unable to predict the occurrence of priming effects because we lack a mechanistic understanding of the processes that regulate priming (Bradford et al., 2008; Billings et al., 2010).

Element stoichiometry constraints the soil microbial community responsible for decomposition; therefore, soil microbes must obtain nutrients from the soil to utilize additional C inputs (Sterner and Elser, 2002; Hungate et al., 2003; Cleveland and Liptzin, 2007; Reed et al., 2011). Support for this concept comes from recent meta-analyses that show inverse relationships between SOM decomposition rates and nutrient availability (van Groenigen et al., 2006; Hungate et al., 2009). Two hypotheses have suggested that priming is controlled by either nutrient availability alone (Kuzyakov et al., 2000) or the response of microbial community structure to nutrient limitation (Fontaine et al., 2003, 2011). The mining of nutrients from SOM by soil organisms is a commonly described mechanism of the priming effect, but the extent to which soil microbial community structure governs the magnitude and direction of priming is uncertain.

A major impediment to the elucidation of priming effect mechanisms is methodological. For instance, Fontaine et al. (2003) proposed a model of SOM dynamics in which the addition of labile C to soil with low nutrient availability could favor slow growing, "*K*-strategist" microbial groups that are able to mine nutrients in SOM

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over fast growing, "*r*-strategist" microbial groups that feed on labile C and nutrients in the soil solution (Fontaine et al., 2003). Yet classifying the soil microbial community into *K*- and *r*-strategists, while possible (Fierer et al., 2007), has remained an elusive goal. Even studying relationships between priming and soil nutrient availability has proven challenging. Fertilizer additions to soil may not adequately simulate nutrients embedded in complex soil organic matter. In order to overcome this limitation, Bradford et al. (2008) advocated studying SOM dynamics along naturally occurring gradients of nutrient availability. Furthermore, most recent studies have only considered relationships between nitrogen (N) and SOM dynamics despite the potential importance of phosphorus (P) to SOM stability, especially in P-limited ecosystems (Cleveland and Townsend, 2006; Cleveland et al., 2006).

Primary succession substrate age gradients may be useful model systems for quantifying mechanisms of SOM dynamics after the addition of exogenous C. Substrate age gradients minimize the variability of soil forming factors, other than time, that may also influence priming effects (climate, parent material, topography, and vegetation; Jenny, 1941). Furthermore, substrate age gradients provide a naturally occurring gradient of soil nutrient availability. As substrate age increases, atmospherically derived nutrients such as C and N progressively increase with soil age to a maximum and then decline in a phase known as retrogression (Peltzer et al., 2010). The retrogressive phase is ascribed to a shift from N to P limitation because rock-derived nutrients such as P are slowly eroded or complexed into unavailable forms (Walker and Syers, 1976; Vitousek and Farrington, 1997; Richardson et al., 2004; Peltzer et al., 2010). Substrate age gradients may also have natural gradients of soil texture (Selmants and Hart, 2008), which can influence SOM dynamics, because SOM can become physically and chemically protected from decomposition by clay particles (Oades, 1988; Lützow et al., 2006). However, no studies to date have measured priming effects in soil from primary succession substrate age gradients, though studies have explored SOM dynamics (Torn et al., 2005) and nutrient limitation of SOM decomposition (Reed et al., 2011) on the Long Substrate Age Gradient in Hawaii (Crews et al., 1995).

We used soil from the Substrate Age Gradient of Arizona (SAGA) to explore mechanisms of the priming effect. Specifically, our goal was to determine if one of the two hypotheses previously discussed (nutrient availability vs. microbial community structure) was more clearly dominant in explaining priming effects. The SAGA, located in semiarid northern Arizona, USA, represents an ideal model system with which to test these hypotheses, for it is well-constrained and has a demonstrated retrogressive gradient of soil C and N, and P availability consistent with the Walker and Syers (1976) model of ecosystem development (Selmants and Hart, 2008, 2010). The silt and clay soil fraction and soil water holding capacity both increase with substrate age across the SAGA (Selmants and Hart, 2008). While little is known about the microbial community structure among the SAGA sites, pools of C change dramatically (Table 1), and the size and composition of the microbial community have previously been suggested as a mechanism explaining other processes observed at the SAGA (Sullivan et al., 2012). We hypothesized that soil nutrient availability would have stronger relationships with priming than microbial community structure, and that priming would follow a U-shaped pattern with increasing substrate age: demand for N (at the youngest substrate age) and P (at the oldest substrate age) after C addition would result in positive priming at the extremes of substrate age. In contrast, we hypothesized that we would observe negative priming at intermediate substrate ages, where the microbial demand for N and P after C additions would be less severe.

2. Methods

2.1. Study sites

The SAGA sites are described in detail by Selmants and Hart (2008). The SAGA is comprised of four sites of varying age within the San Francisco Volcanic Field located in northern Arizona, USA. Soils from all sites are derived from substrates originating from monogenetic volcanic cinder cones that erupted 0.93 ky, 55 ky, 750 ky, and 3000 ky before present. The United States Department of Agriculture Soil Taxonomic subgroups are, in order of increasing substrate age: Typic Ustorthents, Typic Durustands, Typic Argiustolls, and Typic Haplustalfs. All sites are located at similar elevation and experience similar climate (Selmants and Hart, 2008, 2010). Overstory vegetation at all sites is dominated by piñon pine (*Pinus edulis* Engelm.) and one-seed juniper (*Juniperus monosperma* Engelm.) woodland, with canopy interspaces at the three oldest sites dominated by blue grama (*Bouteloua gracilis* (Wild. ex Dunth) Lag. ex Griffiths). At the 0.93 ky-old site, canopy interspaces were dominated by woody shrubs (*Fallugia paradoxa* (D. Don) Endl. ex Torr. and *Rhus trilobata* Nutt.) rather than *B. gracilis*. The climate during the growing season of northern Arizona typically consists of warm, dry early summers, followed by a warm, wet late-summer "monsoon" precipitation pattern (Sheppard et al., 2002) during which time net primary productivity is greatest (Dore et al., 2010).

2.2. Soil incubation

In early August, 2009, we collected soil from the middle of nine canopy interspaces at all sites. Canopy interspaces were a minimum of 10 m diameter between the canopy edges. Samples at each canopy interspace consisted of a composite of five subsamples collected with a 2-cm diameter soil sampler (Oakfield Apparatus, Inc., Oakfield, WI, USA) from 0 to 10 cm mineral soil depth near the center of each canopy interspace. Soils were kept cool (4 °C) during transport to the laboratory, where they were sieved to 2 mm and air-dried. We divided the quantity of soil from each canopy interspace in half, with one half to be an unamended control and the other half to be amended with C in the form of glucose. Before the incubation began,

Table 1
Pools of dissolved organic carbon (DOC), soil total nitrogen (N), total carbon (C) to nitrogen ratio, soil total phosphorus (P), and tree basal area at the four sites that comprise the Substrate Age Gradient of Arizona.

Substrate age (ky)	DOC (mg kg^{-1})[a]	Soil total N (g kg^{-1})[b]	Total C:N[b]	Soil total P (g m^{-2})[c]	Microbial biomass C (mg kg^{-1})[c]	Total tree basal area (m^2 ha^{-1})[d]
0.93	10.8	0.5	18.4	375	15.7	5.7
55	20.4	1.1	11.6	250	85.2	11.6
750	28.3	2.0	13.9	165	581.7	20.7
3000	16.8	1.3	12.5	150	335.3	18.3

[a] B.W. Sullivan, unpublished data, extracted with 0.5 M potassium sulfate.
[b] Selmants and Hart (2008).
[c] Selmants and Hart (2010).
[d] Looney et al. (2012).

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we rewetted the soil to −33 kPa matric potential, as described by Haubensak et al. (2002). We allowed rewetted soils to equilibrate at lab temperature (20 °C) for 3 days prior to incubation.

After the 3-day pre-incubation period, approximately 90 g of wetted soil was measured into 120-ml polyethylene specimen cups, which were then placed in Mason jars with 100 mL of water in the bottom to minimize soil water loss during the course of the incubation. Immediately before sealing the Mason jars, we added uniformly labeled, 99 atom% ^{13}C-glucose to the amended soil in a quantity of C equal to 42% of the microbial biomass C of interspace soils at each site, as measured by chloroform–fumigation–extraction (Table 1; Selmants and Hart, 2008). Measuring the quantity of C added to soil relative to the size of the microbial biomass pool provides a useful means of inter-study comparisons of the priming effect (Blagodatskaya and Kuzyakov, 2008). The Mason jars were sealed with lids fitted with butyl rubber septa, and kept closed between sampling periods. The headspace of the Mason jars was ~0.885 L. The jars were incubated in the dark at 20 °C. Repeated measures analysis of variance showed the gravimetric water contents of the samples did not vary with time over the course of the incubation (data not shown).

For soils from each site, headspace gas samples were randomly sampled from 3 pairs of jars (from a total of 9 pairs of jars) on Day 1, 3, 5, 7, 14, 28, 49, 70, 91, and 112 after the incubation began. To measure soil CO_2 and N_2O evolution, replicate 15 mL samples of headspace gas was removed from the jar headspace and placed in 12 mL evacuated Exetainer vials (Labco Ltd., Buckinghamshire, UK). Immediately afterward, all jars (including those not sampled during that period) were flushed with compressed air and then resealed. Gas flux was calculated from the net accumulation of CO_2 and N_2O in the headspace (relative to control jars that contained no soil) during the sampling period. The CO_2 concentration of the compressed air was measured immediately after each flushing, and was accounted for in flux measurements. One of the replicate 15 mL gas samples was analyzed on a gas chromatograph (Agilent 6890, Palo Alto, CA, USA) equipped with Haysep Q 60/80 and Porapack Q 60/80 packed columns fitted to a flame-ionization detector with a methanizer for measuring CO_2 concentrations, and an electron-capture detector for measuring N_2O concentrations. The other replicate was sent to the Stable Isotope Facility at the University of California, Davis, where samples were measured for ^{13}C–CO_2 using a SerCon Cryoprep TGII trace gas concentration system interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (SerCon Ltd., Cheshire, UK).

We measured changes in N_2O concentrations in the sample headspace to partially quantify N gas loss during nitrification and denitrification. Gaseous losses of N from soil have been used in other studies to infer the strength of soil N limitation (Hall and Matson, 2003; Davidson et al., 2007). Relatively higher N_2O production has been associated with relatively greater availability of N and less N limitation to microbes and plants (Davidson and Seitzinger, 2006). Similarly, N_2O consumption may be an indication of low N availability (Chapuis-Lardy et al., 2007).

On Days 7, 49, and 112 after the incubation began, we destructively sampled three glucose-amended samples and three unamended samples. Each sample was measured for gravimetric water content, inorganic N concentration (ammonium (NH_4^+) plus nitrate (NO_3^-)), available P (as resin-extractable PO_4^{3-}), and total C and N analysis. We measured microbial community composition on Days 7 and 112.

Inorganic N concentration was measured by extracting 10 g of soil with 50 mL of 2 M KCl, shaking for 1 h on a mechanical reciprocating shaker, and filtering through Whatman no. 1 filter paper. We froze the filtered extracts for later analysis of NH_4^+ and NO_3^- pools using a QuickChem 8000 Flow Injection Autoanalyzer (Lachat Instruments, Loveland, CO, USA). Inorganic N concentration was calculated as the sum of NH_4^+ and NO_3^- pools.

We determined soil available P using anion exchange membrane (AEM) strips (10 × 50 mm; Ionics, Inc., Watertown, MA, USA), which is the first step of the Hedley et al. (1982) sequential P extraction procedure (modified by Tiessen and Moir, 2008). Approximately 1 g of moist soil was placed in a 50 mL centrifuge tube with two AEM strips and 30 mL of deionized water, and the solution was rotated on an overhead shaker for 16 h at 30 rpm. We then removed and rinsed the AEM strips with deionized water, placed them in a clean 50 mL centrifuge tube, added 20 mL of 0.5 M HCl, and again rotated the solution for 16 h. The supernatant was frozen and later analyzed for PO_4^{3-}–P on a QuickChem 8000 Flow Injection Autoanalyzer.

We measured soil total N, organic C concentrations and ^{13}C enrichment by oven drying a ~50 mg of each sample at 70 °C, grinding it finely, and analyzing for $^{13}C/^{12}C$ isotopic ratios and total C and N using an elemental analyzer (Carlo Erba NC 2100, CE Instruments, Milan, Italy) interfaced with an isotope ratio mass spectrometer (Thermo-Finnigan Delta Plus XL, Thermo-Electron Corp., Bremen, Germany) isotope ratio mass spectrometer at the Colorado Plateau Stable Isotope Laboratory (http://www.mpcer.nau.edu/isotopelab). The basalt-derived soils at SAGA sites have previously been shown to have little to no inorganic C (Selmants and Hart, 2008).

On Days 7 and 112 of the incubation, we measured PLFA concentration and incorporation of uniformly labeled ^{13}C-glucose using compound-specific isotopic analysis of fatty acids by gas chromatography–isotope ratio mass spectrometry (Meier-Augenstein, 2002). Phospholipids were extracted from 5 g freeze-dried soil using chloroform and methanol. The fatty acids were then separated into glycolipids, neutral lipids, and phospholipids using silicic acid chromatography. Phospholipids were dissolved in hexane and methylated to convert the PLFAs into fatty acid methyl esters (FAMEs). Two standards (13:0 and 19:0) were added before the methylation step. The abundance and ^{13}C isotope ratios of FAME compounds were measured at the University of California-Davis Stable Isotope Facility using a Varian gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) with a FactorFour VF-5ms column (30 m × 0.25 mm ID, 0.25 micron film thickness). Once separated, FAMEs were quantitatively converted to CO_2 in an oxidation reactor at 950 °C. The resultant CO_2 was then analyzed for ^{13}C isotope ratios with isotope ratio mass spectrometry.

The nomenclature we use for PLFA biomarkers is: total number of C atoms, followed by a colon and the number of double bonds, followed by the position (ω) of the double bond measured from the methyl end of the compound. Prefixes *a* and *i* denote anteiso- and iso-branching, respectively. *Cis* and *trans* isomers are denoted by c and t, respectively. The prefix cy denotes cyclopropyl fatty acids; 10Me is a methyl group on the tenth carbon atom from the carboxyl end of the compound. The PLFA compounds 10Me16:0, 10Me17:0, and 10Me18:0 identified actinobacteria (Kroppenstedt, 1985; Brennan, 1988; Zelles et al., 1994); the compounds a15:0, i15:0, and i16:0 identified gram-positive bacteria (O'Leary and Wilkinson, 1988; Frostegard and Bååth, 1996); the compounds c16:1ω9c/7c, c18:1ω9t/7c, cy17:0, and cy19:0 identified gram-negative bacteria (O'Leary and Wilkinson, 1988; Zelles et al., 1994; White et al., 1996); the compounds c18:2ω6c and 16:1ω5c identified fungi (Nordby et al., 1981; Frostegård et al., 1993; Zelles, 1997).

2.3. Calculation of priming effect, PLFA C utilization, and microbial biomass

By using ^{13}C labeled glucose, we were able to determine the fraction of soil- and glucose-derived C in CO_2 and into individual

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PLFA biomarkers using a two-pool mixing model in which the source pools were glucose and native soil C (e.g., Waldrop and Firestone, 2004; Fry, 2007). We calculated the instantaneous priming effect (PE; mg CO_2–C kg soil^{-1} h^{-1}) by subtracting the flux of soil-derived C in glucose-amended soil from soil-derived C in unamended soil; we then converted the instantaneous PE measurements to cumulative priming (mg CO_2–C kg soil^{-1}) by multiplying PE by the number of hours since the previous sampling, and summing each scaled PE since the beginning of the incubation. We calculated relative priming as the cumulative primed C divided by soil total C.

We calculated microbial biomass as the sum of the concentration (nmol g oven dry soil^{-1}) of all PLFA biomarkers with peak area greater than 0.5 (C. Yarnes, UC Davis, personal communication). Relative abundance was measured as the concentration of a given biomarker relative to the concentration of all PLFA biomarkers. We calculated the relative glucose incorporation (%) into the biomass of various microbial groups as the quantity of glucose utilized by the group (nmol g soil^{-1}) divided by total glucose in PLFA biomass (nmol g soil^{-1}).

2.4. Effects of C on N and P fluxes and pool sizes

We calculated the effect of glucose addition on fluxes and pool sizes of N and P by calculating a percent effect size:

% Effect size = ((values of flux or pool in glucose amended soil minus values of flux or pool in unamended soil)/values of flux or pool in unamended soil)*100%. We were then able to calculate cumulative percent effect sizes similarly to cumulative priming. We calculated cumulative percent change of soil inorganic N as the cumulative percent increase or decrease of inorganic N.

2.5. Statistical analysis

We used a combination of one way Analysis of Variance (ANOVA) and multifactor ANOVA to measure the effect of C addition on C, N and P pools. To ascertain if cumulative percent glucose addition effects were significant within a substrate age, we used two-tailed *t*-tests to determine if means were different from zero. We used linear, least-squared regression analysis to relate priming to N, P, and microbial biomass pools, and fluxes of N and P. When appropriate, we used Tukey's HSD post-hoc tests to determine relationships between subjects in ANOVA tests.

We used non-metric multi-dimensional scaling (NMS) in the software package PC-ORD version 5.33 (MjM Software, Gleneden Beach, OR, USA) to examine changes in the relative abundance of the total microbial community using the concentration of PLFA biomarkers on Day 112. We repeated the NMS analysis several times using 250 runs each, with Varimax rotation, to obtain the lowest-stress result. Final stress of the ordination was 8.522 with no instability. The ordination was best explained with a two-dimensional solution. Axis 1 and Axis 2 of the ordination were 93.3% orthogonal.

We used multifactor PerMANOVA in PC-ORD software to determine substrate age and glucose addition effects on the relative abundance of the microbial community on Day 112 of the incubation. We used site, treatment, and the site by treatment interaction as factors.

For all statistical analysis, we evaluated statistical significance, a priori, as $P \leq 0.10$. We used this *P* value because the small sample size of this study ($n = 3$) and substantial anticipated variation within sites and treatment could lead to the occurrence of Type I errors at lower *P* values. Though site ages across the SAGA are unreplicated, the use of unreplicated substrate age gradients nonetheless provides important opportunities for the study of soil and ecosystem development and associated biogeochemical processes (Vitousek, 2002; Wardle et al., 2004). Therefore, we used parametric and non-parametric statistics to infer differences among sites. Because of our sampling approach, which provided spatial replication instead of replicating from a composited sample, we can rigorously test differences among the four substrate ages and make stronger inferences than would otherwise be possible that these differences are due to substrate age rather than another factor. However, we acknowledge that our results must be carefully interpreted, and extrapolation to other sites or ecosystems should be avoided.

3. Results

The priming patterns we observed during the course of our 112-day incubation were consistent with "prolonged" priming, rather than other forms of C utilization, such as pool substitution (Blagodatskaya and Kuzyakov, 2008). Therefore, our results focus on the cumulative priming patterns observed after 112 days of incubation. Temporal dynamics are reported in Supplementary material.

3.1. Carbon and SOM dynamics

Glucose amended soils from the three oldest substrate ages had significantly more cumulative CO_2 efflux than unamended controls (55 ky $t = 3.149$, $P = 0.0439$; 750 ky $t = 7.757$, $P = 0.008$; 3000 ky $t = 2.677$, $P = 0.058$), but the effect of glucose amendment on cumulative CO_2 efflux did not differ among substrate ages (Fig. 1A). Though glucose addition caused similar patterns of cumulative percent change in CO_2 efflux among sites, the quantity of soil-derived CO_2 that was produced as a result of C addition (the primed C) varied in both direction and magnitude (Fig. 1B). However, within-site spatial variation prevented cumulative priming from being significantly different among substrate ages. This large spatial variability also meant that cumulative priming after 112 days was only significantly different from zero at the 750 ky site ($t = 2.082$, $P = 0.086$). Because each site had different amounts of SOM before glucose addition, mean relative priming (the amount of primed C divided by the amount of soil total C) provides a measure of the relative importance of priming at each substrate age. Mean relative priming declined with substrate age to the 750 ky site, and then increased back to zero at the 3000 ky site (Fig. 1C); but like cumulative priming, relative priming was not significantly different among sites, and relative priming only differed from zero at the 750 ky site. Positive priming at the 0.93 ky site represented increased decomposition of soil total C of ~5%, and negative priming at the 750 ky site represented decreased decomposition of soil total C of ~3%.

3.2. Nitrogen and phosphorus dynamics

Soils that were amended with glucose had less soil inorganic N at the end of the incubation than unamended soil (Table 2; $F = 5.403$, $P = 0.038$). The cumulative percent change in inorganic N caused by glucose addition was significantly lower at the 750 ky site than at the 0.93 ky, 55 ky, or 3000 ky sites (Table 2; $F = 18.944$, $P < 0.001$). The cumulative effect of glucose on the soil inorganic N pool was significantly different from zero at the three youngest substrate ages after 112 days ($t > 1.864$, $P \leq 0.010$). Cumulative priming after 112 days was significantly and positively correlated with the cumulative percent change of soil inorganic N content caused by glucose addition (Fig. 2; $r^2 = 0.47$, $P = 0.01$).

The effects of glucose addition on N_2O fluxes were significantly different between the 0.93 ky site and the three oldest sites (Fig. 1D;

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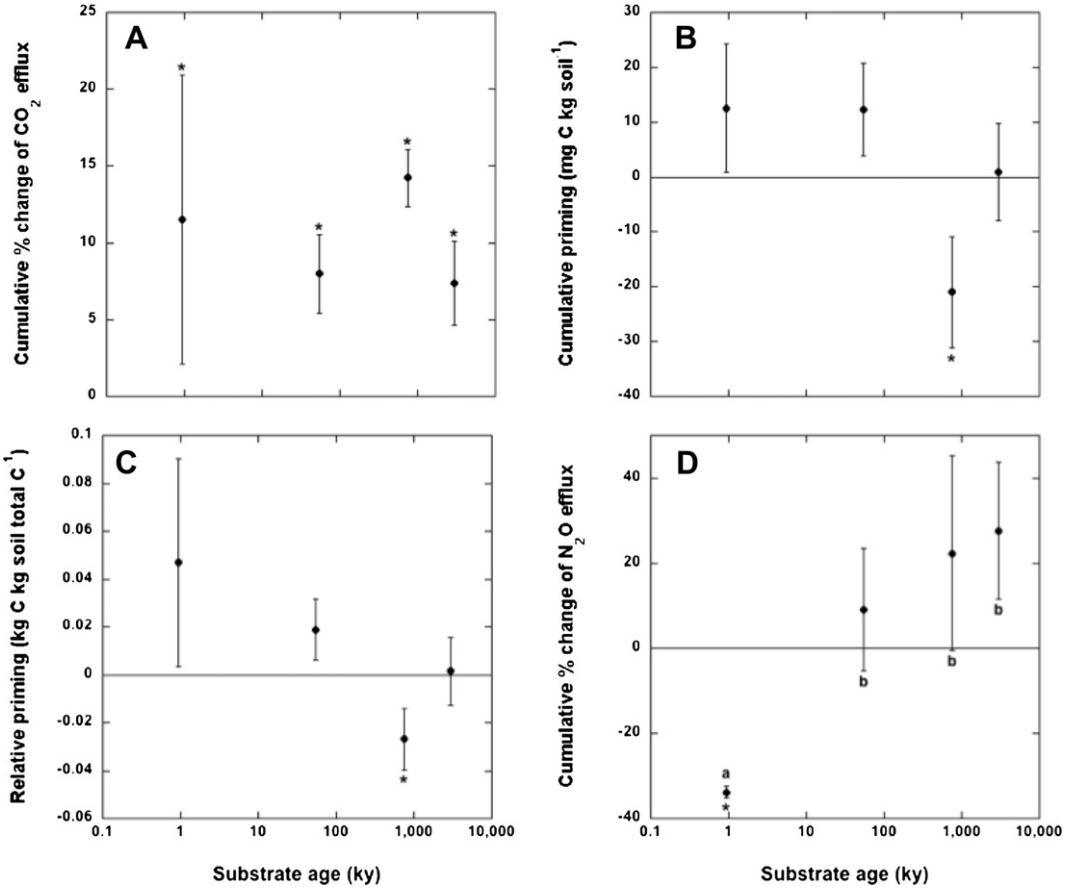

Fig. 1. Cumulative percent change of carbon dioxide (CO_2) efflux (A), cumulative priming (B), relative priming (C) and cumulative percent changes in N_2O efflux (D) resulting from glucose addition during the course of the 112 day incubation at the four substrate ages that comprise the Substrate Age Gradient of Arizona. Relative priming was calculated as the cumulative priming divided by the total soil C pool at each site. Data are presented as means ± one standard error. Asterisks indicate percent changes due to glucose addition that were significantly different from zero; lowercase letters indicate significant differences among sites.

$F = 3.123$, $P = 0.088$). The 0.93 ky site had significantly less N_2O efflux in glucose-amended soil than unamended soil on Day 112 ($t = 23.514$, $P < 0.001$). But importantly, this difference was due to a strong negative effect of glucose addition on N_2O efflux on Day 3 of the incubation; between days 5 and 112, glucose-amended soil at the 0.93 ky site actually had higher N_2O fluxes than unamended soil, resulting in a positive trend through time (Supplementary Data). The 0.93 ky site was the only site with a positive trend in the cumulative percent change of N_2O efflux over time.

Table 2
Mean (±1 standard error) cumulative changes to inorganic nitrogen (N), available phosphorus (P), and microbial fungal-to-bacterial biomass ratio after 112 days of incubation following glucose addition to soil from each of the four substrate ages that comprise the Substrate Age Gradient of Arizona. Asterisks denote cumulative percent changes of the variable of interest that were significantly different from zero ($P \leq 0.10$) after 112 days. Within a sampling date, significant differences among substrate ages are denoted with different letters. If no letters are denoted, there was no significant difference among sites.

Substrate age (ky)	Cumulative % change in inorganic N	Cumulative % change in available P	Cumulative % change of microbial fungal–bacterial-biomass ratio
0.93	−23.3 (10.8) a*	−54.0 (28.7)*	−6.3 (3.3)*
55	−24.6 (13.2) a*	−2.2 (18.5)	−9.7 (13.2)
750	−94.3 (0.9) b*	−29.2 (8.6)*	9.5 (5.4)
3000	−16.3 (9.5) a	0.6 (14.9)	−0.2 (5.5)

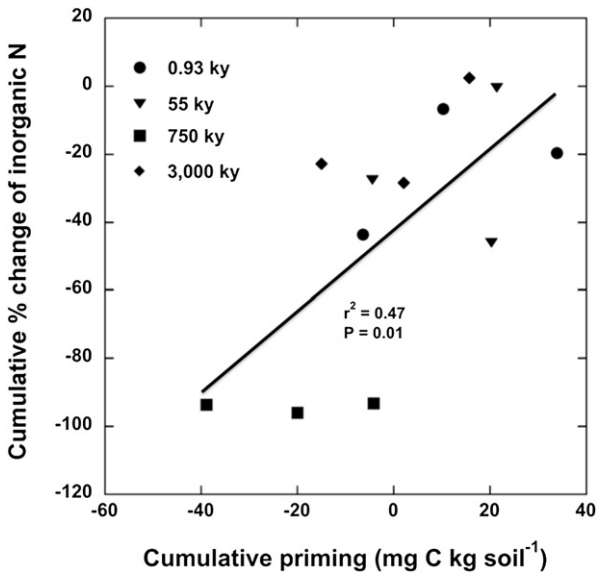

Fig. 2. Cumulative percent change of the soil inorganic nitrogen (N) pool caused by glucose addition in soil from the four sites that comprise the Substrate Age Gradient of Arizona (SAGA) plotted as a function of cumulative priming in the same soils after 112 days of incubation.

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The effect of glucose on available P concentrations did not vary significantly between substrate ages (Table 2). At both the 0.93 ky and 750 ky sites, cumulative glucose addition effects on available P after 112 days were significantly less than zero ($t = -1.881$, $P = 0.100$ and $t = -3.382$, $P = 0.039$, respectively). Cumulative glucose addition effects on available P were negligible at the 55 ky and 3000 ky sites, and there were no significant relationships between priming and the effect of glucose addition on available P.

3.3. Microbial community dynamics and glucose utilization

The microbial community of both glucose-amended and unamended soil was significantly different at the 0.93 ky site than the three older substrate ages (Fig. 3; $F = 5.264$, $P = 0.001$). However, glucose addition had no significant or consistent effect on the relative abundance of the total microbial community (Fig. 3), though glucose addition caused the microbial community structure to vertically shift up Axis 2 of the 112 day NMS ordination at the three older substrate ages (Fig. 3). All functional groups (actinobacteria, other gram positive bacteria, gram negative bacteria, and fungi) were more highly correlated with Axis 2 of the ordination than Axis 1. Combined, the NMS ordination axes explained 95.5% of the variance of the microbial community. Axis 1 of the NMS ordination was more strongly correlated with soil available N and P pools, total C and N pools, and C:N ratio than Axis 2; percent N was most strongly correlated with Axis 2 ($r^2 = 0.389$).

Glucose addition significantly changed the microbial biomass in an uni-modal relationship with substrate age (Fig. 4A; $F = 5.025$, $P = 0.032$). Glucose addition significantly reduced microbial biomass relative to unamended controls at the 0.93 ky site ($t = 3.364$, $P = 0.039$) and the 55 ky site ($t = 2.777$, $P = 0.054$). Glucose addition did not result in a statistically significant change in the size of the microbial biomass relative to unamended controls at the 750 ky and 3000 ky sites. Changes of microbial biomass as a result of glucose addition were significantly and negatively related to changes in relative priming (Fig. 5; $F = 7.726$ $P = 0.020$). The predictive power of this relationship ($r^2 = 0.436$) improved substantially ($r^2 = 0.647$) when two points are removed from consideration that both had the largest decrease in microbial biomass (−50 to −60%), but very different relative priming effects.

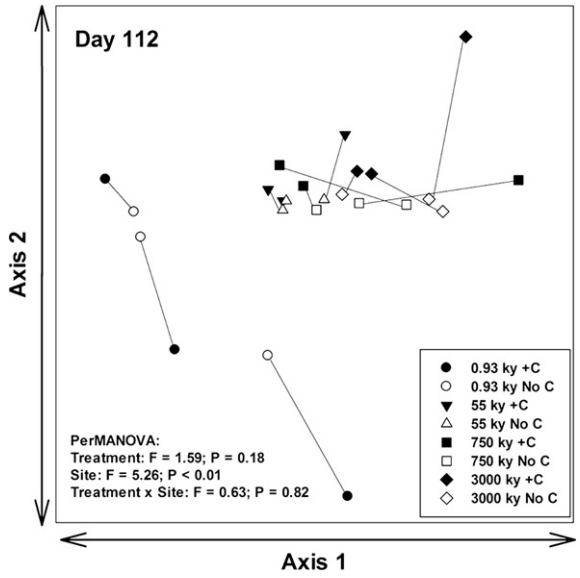

Fig. 3. Non-metric multidimensional scaling ordination of microbial community relative abundances in glucose-amended (shaded symbols) and unamended (unshaded symbols) soil for each of the four substrate ages that comprised the Substrate Age Gradient of Arizona after 112 days of incubation. PerMANOVA statistical analysis indicated no significant treatment effect, though there was a significant difference in relative abundance among substrate ages.

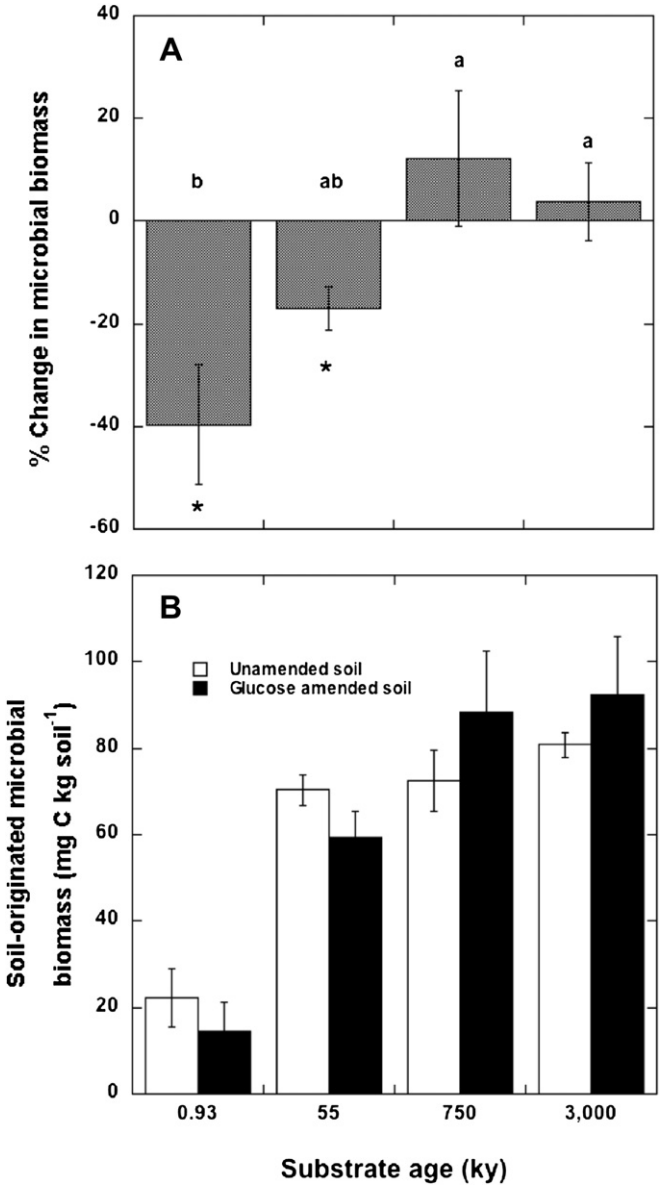

Fig. 4. Percent change in microbial biomass (as measured by total phospholipid fatty acid concentrations) caused by glucose addition (A) and soil-originated microbial biomass (B) in unamended (white bars) and glucose-amended (dark bars) at each of the four substrate ages that comprised the Substrate Age Gradient of Arizona after 112 days of incubation. Significant differences in microbial biomass among substrate ages are denoted by different letters lowercase letters. Asterisks denote percent changes in microbial biomass that were significantly different from zero. Vertical lines represent ± one standard error of the mean.

By Day 112, glucose-amended soil from the 750 ky site had a higher fungal to bacterial biomass ratio than did unamended controls (Table 2; $F = 6.303$, $P = 0.040$). Glucose addition caused a decrease of fungi relative to bacteria of −6% at the 0.93 ky site and −9% at the 55 ky site ($F = 23.391$, $P < 0.001$).

The actinobacteria and fungi microbial groups incorporated the least glucose during the course of the incubation, whereas gram negative bacteria incorporated the most glucose (Fig. 6). Relative glucose incorporation varied significantly by substrate age ($F = 3.461$, $P = 0.028$).

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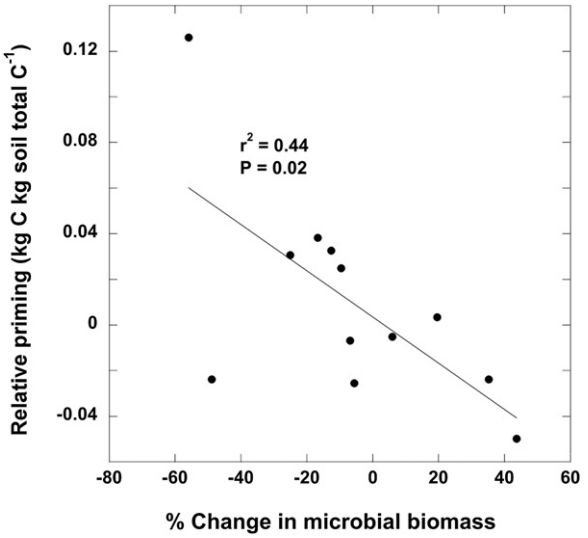

Fig. 5. Relative priming caused by glucose addition after 112 days of incubation plotted as a function of the percent change in microbial biomass (measured by total phospholipid fatty acid concentrations) after 112 days of incubation. Relative priming was calculated as the cumulative priming divided by the total soil C pool at each site.

4. Discussion

With this study, we sought to measure patterns of priming among sites differing in substrate age, and determine if nutrient availability or microbial community dynamics were responsible for the patterns of priming we observed. To our knowledge, this study is the first to measure different magnitudes and directions of priming among sites varying in substrate age, though Milcu et al. (2011) recently measured priming at a secondary successional gradient. Though the effects were small in absolute values and the associated error was large (because of our within-site spatial replication), we observed positive priming, negative priming, and no net priming effects as a function of three million years of soil development.

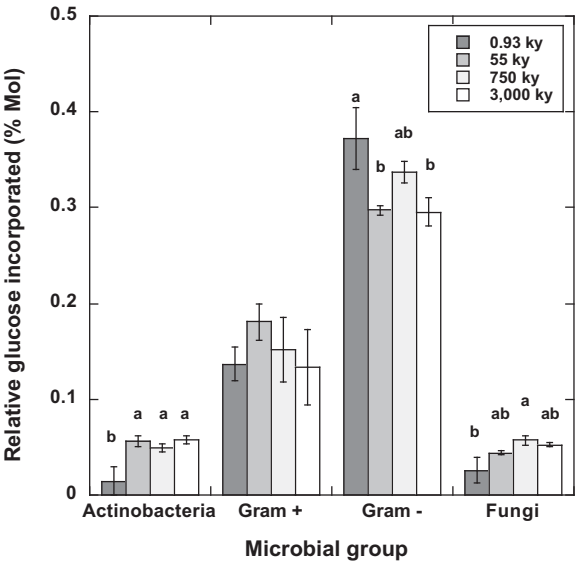

Fig. 6. Relative incorporation of glucose into the following four major microbial groups: actinobacteria, Gram positive (Gram +) bacteria, Gram negative (Gram −) bacteria, and fungi at each of the four sites that comprised the Substrate Age Gradient of Arizona after 112 days of incubation. Within a microbial group, significant differences in relative substrate incorporation among substrate ages are denoted by different lower-case letters. Vertical lines represent ± one standard error of the mean.

The pattern of generally positive priming occurring at the two youngest substrate ages, and negative or no net priming occurring at the two oldest substrate ages could be caused by at least two of the strong gradients observed among the SAGA sites: the strong soil textural gradient and the strong nutrient gradients. Fine-textured soil has been associated with greater SOM stocks (Arrouays et al., 2006) and greater SOM stability as a result of both chemical and physical characteristics (Duchaufour, 1976; Oades, 1988; Lützow et al., 2006). The patterns we observed are consistent with the relationship of soil texture and SOM stability described by Arrouays et al. (2006); the oldest soil with the finest texture experienced either negative or no net priming of SOM. However, in this study as in others (Oades, 1988), soil texture is also associated with changes in other factors that influence SOM stability, and our results highlight the important role of soil nutrients in SOM dynamics. The Walker and Syers (1976) model, which suggests that old soils are more limited in rock-derived nutrients such as P, and was expanded to suggest that young soils are more limited in atmospheric-derived nutrients such as N (Vitousek and Farrington, 1997; Richardson et al., 2004; Wardle et al., 2004; Peltzer et al., 2010), would indicate that positive priming was observed at the sites with the lowest soil N availability (Selmants and Hart, 2008).

Our results provide several pieces of evidence that in this semiarid ecosystem, N, but not P, availability plays a much stronger role in mediating priming effects than does the soil microbial community. First, more inorganic N was immobilized in glucose-amended soils than unamended soils at the 750 ky site, where priming was most negative. Second, when all four sites were combined, greater priming was correlated with less net N immobilization, suggesting more N released from native SOM by priming. Third, more N_2O was produced in glucose-amended soil than unamended soil at the 0.93 ky site after the first three days of the incubation, where priming was the most positive (Supplementary Data). On the other hand, the lack of correlation between cumulative priming and available P would suggest that priming was not caused by a demand for P from native organic matter. For instance, after 112 days, the 0.93 ky site and the 750 ky site both had significantly less available P in soils to which glucose was added than unamended soils, despite having opposite mean cumulative priming effects. Taken together, these results are consistent with the hypothesis that SOM is decomposed to obtain recalcitrant N within the SOM (Kuzyakov, 2002; Fontaine et al., 2011).

We were unable to provide evidence in support of the hypothesis that changes in the soil microbial community following C addition are responsible for priming (Fontaine et al., 2003). We were unable to concretely identify "functional" groups (such as *K*- and *r*-strategists, Fontaine et al., 2003; Fierer et al., 2007) responsible for the priming patterns we observed. The strongest pattern we observed was that the ratio of fungal to bacterial biomass was higher at the 750 ky site than any other after 112 days, and this site had the most negative priming. This result seems to contradict evidence that C addition increased the abundance of fungi relative to the whole microbial community and that suggested fungi are responsible for priming (Fontaine et al., 2011). On the contrary, Bird et al. (2011) posit that Gram positive bacteria are responsible for long-term rhizosphere priming in a California grassland ecosystem. Yet our results fail to conclusively establish that either bacteria or fungi are responsible for priming, or that priming was related to shifts in these functional groups. At the two youngest substrate ages that experienced the greatest cumulative priming after 112 days, glucose-amended soil had lower fungal to bacterial biomass ratios than unamended controls, suggesting a greater bacterial response to glucose addition, which could suggest a link to priming. However, more glucose was incorporated into gram positive and gram negative bacteria than fungi or actinobacteria, suggesting that

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here bacteria utilized glucose while actinobacteria and fungi utilized SOM.

It is unsurprising that the need for N, rather than P, would cause the general soil microbial community to mineralize SOM, for N is mineralized during the decomposition of C compounds whereas P is 'mineralized' by the cleaving of an ester bond, which does not require the breakdown of the C skeleton of SOM (McGill and Cole, 1981). In fact, N, not P, mineralization during priming was exactly the pattern observed during secondary succession by Milcu et al. (2011) in a very different ecosystem. But because recent evidence has indicated that P may limit C decomposition (Cleveland and Townsend, 2006; Cleveland et al., 2006), and because the SAGA represents a gradient of P availability (Selmants and Hart, 2010), we expected to observe a relationship between priming and P availability. Phosphorus availability is more difficult to measure than nitrogen; therefore, we suggest three non-exclusive mechanisms that could explain a lack of P mineralization during priming. First, it is possible that sufficient P is available at all substrate ages to meet microbial demands to maintain C:P stoichiometry after C addition (Cleveland and Liptzin, 2007). Selmants and Hart (2008) found that the easily weatherable (and therefore labile) calcium-occluded P pool still comprised roughly 50% of the surface soil P pool after three million years. In contrast, at the humid Franz Josef substrate age gradient, the calcium-occluded P pool disappeared by 22 ky after pedogenesis (Walker and Syers, 1976). Second, as P was mineralized, it may have been sorbed to calcium (P. Selmants, unpublished data) or converted to organic P. Our measurements of the fast-cycling, labile PO_4^{3-} pool would therefore have failed to measure changes in this organic P pool. Third, the lack of plant root or mycorrhizal activity in our incubations may have reduced rates of organic P mineralization to PO_4^{3-}. Future studies exploring the relationship between priming and P ought to consider more thorough investigations of P pools using other methods, such as the Hedley et al. (1982) sequential extraction method or NMR of the organic P fraction (e.g., Turner et al., 2007), or should assess changes in phosphatase enzyme activity (Sinsabaugh et al., 1999).

In addition to these patterns, our study elucidated other important facts pertaining to priming effects in soil. Though we used glucose in lower quantities than many other laboratory studies (Blagodatskaya and Kuzyakov, 2008), we measured all three possible patterns of priming. In a meta-analysis, Blagodatskaya and Kuzyakov (2008) recommended that priming studies standardize the quantity of C added by the C in microbial biomass, but also suggested that the presence and magnitude of a priming effect may be caused by the amount of labile C added to soil. Our results clearly show that priming effects can vary in direction and magnitude at the same relativized C concentration. In the present study, though the total mass of glucose we added to soil varied among substrate ages, at each site the glucose was 42% of the total microbial biomass, measured by chloroform–fumigation–extraction (Haubensak et al., 2002).

Pool substitution, or the replacement of ^{12}C in microbial biomass with ^{13}C, rather than the decomposition of SOM, has been identified as a source of error in priming studies using isotopically labeled substrates (called "false priming" by Fontaine et al., 2011). We maintain that our results were the result of actual decomposition of SOM for several reasons. We observed no significant differences in soil-originated microbial biomass between C-amended and unamended soils (Fig. 4B), and a significant difference in soil-originated microbial biomass has been previously used as a test of "false priming" (Fontaine et al., 2011). We also successfully recovered 83–93% of all glucose we added (data not shown), despite using small concentrations relative to many other priming studies (Blagodatskaya and Kuzyakov, 2008). Hence, we conclude that the difference between $^{12}CO_2$ fluxes we measured in glucose-amended and unamended soils were likely the result of changes in the size of the SOM pool and not the result of pool substitution.

5. Conclusions

Given the size of the global soil C pool (1200 Pg C), even small changes in SOM decomposition rates can have large consequences on atmospheric CO_2 concentrations (Houghton, 2007). Though we measured small absolute cumulative priming rates, priming ranged from +5% to −3% of the soil total C pool within a site over a 112 day laboratory incubation at optimal water content. Clearly, it is critical that we improve our mechanistic understanding of priming and its influence on the global C balance, for we must identify the places where SOM is more or less stable in order to accurately predict SOM stocks in the future. This research takes a step in that direction in two ways. Based on our results, biogeochemical models of priming should focus more strongly on soil nutrient availability than on a metric of soil microbial community structure. Furthermore, N, rather than P, appears to be the dominant control of priming, yet this result must be validated in other ecosystems, notably tropical ones, where P limitation dominates ecosystem processes (Vitousek et al., 2010).

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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.2012.12.007.

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