

SHORT COMMUNICATION

Linking soil bacterial biodiversity and soil carbon stability

Rebecca L Mau^{1,2}, Cindy M Liu^{3,4}, Maliha Aziz³, Egbert Schwartz^{1,2}, Paul Dijkstra^{1,2}, Jane C Marks^{1,2}, Lance B Price³, Paul Keim^{2,3,4} and Bruce A Hungate^{1,2}

¹Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ, USA; ²Center for Ecosystem Science and Society (EcoSS), Northern Arizona University, Flagstaff, AZ, USA; ³Translational Genomics Research Institute (TGen North), Flagstaff, AZ, USA and ⁴Microbial Genetic and Genomics Center (MGGen), Northern Arizona University, Flagstaff, AZ, USA

Native soil carbon (C) can be lost in response to fresh C inputs, a phenomenon observed for decades yet still not understood. Using dual-stable isotope probing, we show that changes in the diversity and composition of two functional bacterial groups occur with this ‘priming’ effect. A single-substrate pulse suppressed native soil C loss and reduced bacterial diversity, whereas repeated substrate pulses stimulated native soil C loss and increased diversity. Increased diversity after repeated C amendments contrasts with resource competition theory, and may be explained by increased predation as evidenced by a decrease in bacterial 16S rRNA gene copies. Our results suggest that biodiversity and composition of the soil microbial community change in concert with its functioning, with consequences for native soil C stability.

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Substrate inputs can stimulate decomposition of native soil organic carbon (SOC; Kuzyakov *et al.*, 2000), a phenomenon known as the ‘priming effect’ (Kuzyakov, 2010), and is considered large enough to influence ecosystem C balance (Wieder *et al.*, 2013). Two functionally distinct groups of microorganisms are postulated to mediate priming: one that grows rapidly utilizing labile C, and one that grows slowly, breaking down recalcitrant SOC (Fontaine *et al.*, 2003; Blagodatskaya *et al.*, 2007). However, distinguishing these groups is technically challenging. Here, we used dual-stable isotope probing with ¹³C-glucose and ¹⁸O-water to identify bacteria in these two groups growing in response to single and repeated pulses of glucose. Organisms that utilize labile C for growth assimilate both ¹³C-glucose and ¹⁸O-water into their DNA, whereas organisms that grow using SOC incorporate only ¹⁸O-water. Differential isotope incorporation leads to a range of DNA densities separable through isopycnic centrifugation, which can then be characterized by sequencing (Radajewski *et al.*, 2000).

We sequenced fragments of bacterial 16S rRNA genes following single and repeated glucose pulses. We hypothesized that the single pulse of labile C would stimulate growth of opportunistic organisms,

thus immobilizing nutrients and suppressing growth and diversity of the SOC-utilizing community, decreasing SOC decomposition (negative priming), a response analogous to that observed in plant communities in response to chronic N additions (Tilman, 1987; Clark and Tilman, 2008). We hypothesized that multiple glucose additions would stimulate growth of a more diverse bacterial community, including more native SOC-utilizing organisms that possess enzymes to decompose recalcitrant compounds, causing positive priming (Fontaine *et al.*, 2003; Kuzyakov, 2010).

Soil from a ponderosa pine ecosystem was amended weekly for 7 weeks with 500 µg C-glucose per gram soil (2.65 atom % ¹³C) in 100 µl deionized water or with 100 µl deionized water ($n=5$). Measurements of $\delta^{13}\text{C}-\text{CO}_2$ and $[\text{CO}_2]$ enabled the partitioning of CO_2 into that derived from added glucose or from native SOC (C_{SOC}):

$$C_{\text{SOC}} = C_{\text{total}} (\delta_{\text{total}} - \delta_{\text{glucose}}) / (\delta_{\text{SOC}} - \delta_{\text{glucose}})$$

where C_{total} is $\text{CO}_2\text{-C}$ from glucose-amended samples, δ_{total} is the $\delta^{13}\text{C}-\text{CO}_2$ from glucose-amended samples, δ_{glucose} is the $\delta^{13}\text{C}$ of the added glucose and δ_{SOC} is the $\delta^{13}\text{C}-\text{CO}_2$ evolved from the non-amended samples. Priming was calculated as the difference between SOC oxidation of the amended and non-amended samples. With this approach, any evolved CO_2 carrying the ¹³C signature of the added glucose is considered respiration of glucose, including ¹³C-labeled biomass and metabolites derived from prior glucose additions. Thus, this approach

Correspondence: BA Hungate, Center for Ecosystem Science and Society, Department of Biological Sciences, Northern Arizona University, Box 5620, Flagstaff, AZ 86011-5620, USA.
E-mail: Bruce.Hungate@nau.edu

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quantifies priming as the oxidation of SOC present at the beginning of the experiment, consistent with many other studies of priming (Cheng *et al.*, 2003; De Graaff *et al.*, 2010).

In a parallel incubation for dual-stable isotope probing, the repeated-pulse samples received unlabeled glucose (500 μg C-glucose per gram soil) for 6 weeks while the non-amended and single-pulse samples received sterile deionized water. In week 7, samples received one of four isotope treatments ($n=3$): 97 atom % H_2 ^{18}O (non-amended soil), 99 atom % ^{13}C -glucose and 97 atom % H_2 ^{18}O (single- and repeated-pulse soil), ^{12}C -glucose and 97 atom % H_2 ^{18}O (repeated-pulse soil) or ^{12}C -glucose and H_2 ^{16}O (repeated-pulse soil). After incubating for 7 days, soil was frozen at -40°C . DNA was extracted, separated through isopycnic centrifugation, and two density ranges were sequenced for the bacterial 16S rRNA gene (Supplementary Figure 1): 1.731–1.746 g ml^{-1} (hereafter called the SOC-utilizing community) and 1.759–1.774 g ml^{-1} (hereafter called the glucose-utilizing community).

Amplicons of the V3–V6 16S rRNA region were bar coded with broad-coverage fusion PCR primers and pooled before sequencing on a Genome Sequencer FLX instrument. These sequence data have been submitted to the GenBank database under accession number SRP043371. Data were checked for chimeras (Edgar *et al.*, 2011), demultiplexed and quality checked (Caporaso *et al.*, 2010). Taxonomy was assigned to genus at the $\geq 80\%$ bootstrap confidence level (Cole *et al.*, 2009).

We used the Shannon's diversity index (H'), commonly used in microbial systems (Fierer and Jackson, 2006), to assess changes in microbial diversity. Analysis of variance was used to compare the amount of DNA within densities between isotope treatments (Supplementary Figure 2) and to test the effects of the treatments on the Shannon's diversity (Figure 2) and Pielou's evenness (Supplementary Figure 3) of the active bacterial communities, with *post hoc* Student's *t*-tests, $\alpha=0.05$. PRIMER 6 and PERMANOVA were used to create the nonmetric multidimensional scaling

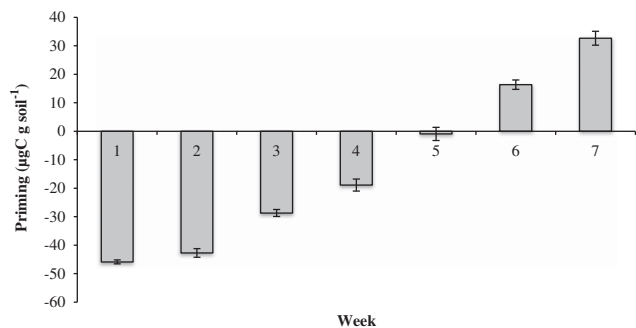


Figure 1 Weekly priming rates calculated as the difference in SOC respired between glucose-amended and non-amended soil ($n=5$).

ordination and to compare bacterial communities between glucose treatments and the two sequenced density ranges.

The single pulse of glucose suppressed SOC oxidation, whereas repeated pulses increased SOC oxidation (Figure 1). Few experiments to date have examined priming in response to repeated substrate amendments (Hamer and Marschner, 2005; Qiao *et al.*, 2014), even though in nature soil receives repeated substrate pulses from litterfall and rhizodeposition. Our results demonstrate the dynamic response of SOC decomposition to repeated labile C inputs.

Dual-stable isotope probing was able to separate the growing bacteria into two groups with distinct DNA densities ($P<0.001$, PERMANOVA; Figure 3a), indicating differential uptake of ^{13}C -glucose and ^{18}O -water. In response to the initial glucose addition, the diversity of the growing glucose- and SOC-utilizing bacterial communities declined compared with the non-amended community ($P<0.001$, *t*-tests; Figure 2), driven by a strong decrease in evenness (Supplementary Figure 3). In the SOC-utilizing community, where DNA was labeled with ^{18}O only, the relative abundance of *Bacillus* increased 4.9-fold compared with the non-amended control to constitute 31.6% of the community (Figure 3b). *Bacillus* survives well under low-nutrient conditions (Panikov, 1995), and is able to synthesize a suite of extracellular enzymes capable of degrading complex substrates (Priest, 1977), traits that are conducive for using SOC for growth. In the glucose-utilizing community, where DNA was labeled with both ^{13}C and ^{18}O , *Arthrobacter* increased 67.7-fold relative to the non-amended control to constitute 75.5% of the growing bacteria (Figure 3b). In culture experiments, *Arthrobacter* can rapidly take up and store glucose for later use (Panikov, 1995) and here we find it dominating the high-density DNA fractions, signifying that it is using the labeled glucose to grow. The increased biomass of *Arthrobacter* may have resulted in greater resource competition, thus reducing the diversity of the growing community, as is frequently found in plant communities (Bakelaar and Odum, 1978; Clark and Tilman, 2008).

After repeated glucose amendments, the diversity of the growing community recovered to non-amendment levels (Figure 2) without strongly dominant organisms (Figure 3b and Supplementary Figure 3). The higher diversity found after repeated glucose pulses may be explained by trophic interactions where predators graze on prey populations that have been enlarged by resource addition, suppressing competition between prey species and causing secondary mobilization of nutrients (Clarholm, 1985). The decrease in total bacterial 16S rRNA gene copies in the repeated-pulse—compared with the single-pulse—treatment (Supplementary Figure 4) supports predation as a potential mechanism explaining the observed diversity increase after repeated glucose pulses.



Figure 2 Shannon's diversity index (H') of the non-amended, single-pulse, and repeated-pulse treatments ($n=3$) in the SOC- (mid-density) and glucose-utilizing (high-density) communities. Treatments with the same letter are not significantly different from each other (Student's t , $\alpha=0.05$).

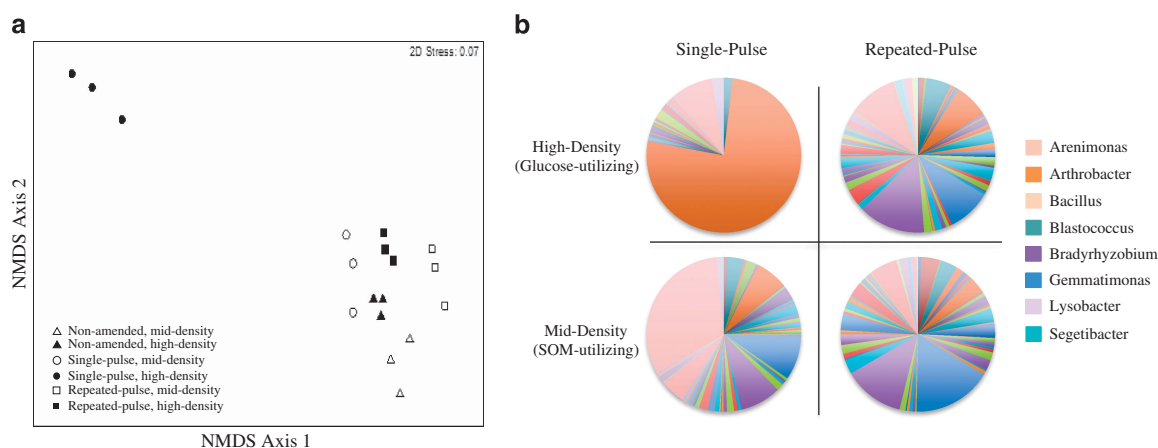


Figure 3 (a) Nonmetric multidimensional scaling ordination showing differences in growing bacterial communities at the genus taxonomic level in the SOC-utilizing (mid-density; open symbols) and glucose-utilizing (high-density; closed symbols) groups of non-amended (Δ), single-pulse (\circ) and repeated-pulse (\square) treatments ($n=3$). (b) Pie charts of genera in the SOC- and glucose-utilizing communities of the single- and repeated-pulse treatments ($n=3$). Genera with relative abundances $>5\%$ are listed in the figure legend.

The recovery of diversity after repeated glucose pulses contrasts with resource competition theory (Tilman, 1987). When chronic additions of a limiting resource are applied, species diversity and evenness typically decrease (Bakelaar and Odum, 1978; Clark and Tilman, 2008) because competitive organisms become dominant. We observed this after the single glucose pulse, but not after repeated pulses. This diversity response may be the result of community shifts facilitated by short bacterial life cycles and the tens to hundreds of generations expected during the 7-week incubation (Behera and Wagner, 1974). In contrast, systems on which most ecological theory is based (for example, plants) might achieve perhaps 20 generations in a multi-decadal field experiment (Bakelaar and Odum, 1978; Clark and Tilman, 2008). With more generations, more community dynamics can occur, including increased resource cascades in which extracellular enzymes, metabolites or lysed cells of one functional group increase substrates for another (Blagodatskaya and Kuzyakov, 2008). Our results highlight the opportunity to test ecological theories in microbial ecosystems (Prosser *et al.*, 2007),

particularly as the short life cycles of microbes makes them well suited for pursuing ecological questions in an evolutionary framework (Jessup *et al.*, 2004).

The priming effect is ubiquitous, yet its drivers remain elusive. Our results suggest that changes in the diversity and composition of the growing bacterial community contribute to priming, and thus that ecosystem properties such as soil C storage may be sensitive to soil microbial biodiversity.

Conflict of Interest

The authors declare no conflict of interest.

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