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SOIL MICROBIOLOGY

Physiological Profiling of Soil Microbial Communities in a Florida Scrub-Oak Ecosystem: Spatial Distribution and Nutrient Limitations

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Abstract Rapid physiological profiling of heterotrophic microbial communities enables intensive analysis of the factors affecting activity in aerobic habitats, such as soil. Previous methods for performing such profiling were severely limited due to enrichment bias and inflexibility in incubation conditions. We tested a new physiological profiling approach based on a microtiter plate oxygen sensor system (Becton Dickinson Oxygen Biosensor System (BDOBS)), which allows for testing of lower substrate addition (i.e., lower enrichment potential) and manipulation of physiochemical assay conditions, such as pH and nutrients. Soil microbial communities associated with a scrub-oak forest ecosystem on Merritt Island Wildlife Refuge in central Florida, USA, were studied in order to evaluate microbial activity in a nutrient poor soil and to provide baseline data on the site for subsequent evaluation of the effects of elevated CO_2 on ecosystem function. The spatial variation in physiological activity amongst different habitats (litter, bulk soil, and rhizosphere) was examined as a function of adaptation to local resources (i.e., water soluble extracts of roots and leaf litter) and the degree of N and P limitation. All the communities were primarily N-limited, with a secondary P limitation, which was greater in the rhizosphere and bulk soil. The litter community showed greater overall oxygen consumption when exposed to litter extracts relative to the rhizosphere or soil, suggesting acclimation toward greater use of the mixed substrates in the extract. Root extracts were readily used by communities from all the habitats with no habitat specific acclimation observed. A priming effect was detected in all habitats; addition of glucose caused a significant increase in the use of soil organic carbon. Response to added glucose was only observed with N and P addition, suggesting that C may be lost to the groundwater from these porous soils because nutrient limitation prevents C immobilization.

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Introduction

Soil organic matter (SOM) is an important global reservoir of carbon, representing over 48% of the active terrestrial surface pool [20]. Microbial processing of this SOM is affected by multiple factors, including altered nutrient inputs and litter deposition rates associated with natural and anthropogenic processes [27]. Estimating *in situ* rates of microbial processing rates via respirometry, including approaches using stable isotopes, is critical to defining ecosystem-level carbon fluxes. Current methods for assessing heterotrophic microbial activity are time consuming and thereby limit the spatial or temporal intensity of sampling. High throughput methods used for estimating the respiration of soil samples, even if not direct estimators of flux, would be valuable for rapid assessment of factors affecting microbial respiration

The BD Oxygen Biosensor System (BD Biosciences, Bedford, MA) [11], a new method of monitoring substrate-induced respiration, provides increased sensitivity and a potentially more realistic estimate of substrate use and nutrient limitation. BDOBS utilizes a ruthenium dye that

fluoresces as dissolved oxygen is depleted in the overlying microbial inoculums, yielding a measurement of O_2 use and, concomitantly, substrate utilization [10, 11]. The BDOBS system is a more appropriate method for examining the soil microbial communities in the Florida scrub-oak ecosystem relative to previously employed microtiter-based methods for assessing substrate utilization (i.e., Biolog plates) because it (1) allows more rapid assessment of the community response (several hours compared to 1–4-day incubations), (2) eliminates the need for extraction of cells from the soil matrix since the dye response is read from the bottom, allowing detection of activity despite the opacity of the soil slurry, (3) allows for 10- to 100-fold lower substrate concentrations due to measurement of dissolved O_2 consumption rather than CO_2 evolution, and (4) allows for manipulation of nutrient availability and pH [10, 11]. The increased sensitivity of the assay allows the use of substrates derived from native materials (e.g., leaf litter and root), thereby providing insight into the acclimation of microbes from a particular habitat to its available substrates. The increased sensitivity also suggests responses may be detectable without substrate amendments, allowing for the estimation of bio-available soil organic matter.

The study site on the Merritt Island National Wildlife Reserve, Florida, is a nutrient-limited, scrub-oak ecosystem with low soil organic matter [17], making it ideal for studies on C dynamics and nutrient limitations. Studies have shown higher abundance of microbes in the litter layer than soil by monitoring bacterial counts [3, 19] and respiration measurements [15]. Albarracin (Master's Thesis, University of Central Florida, 2005) found that there were significantly more culturable cells in litter than soil in the Florida scrub-oak. Microbial attributes, such as biomass [9], capacity for substrate use [13], and respiration [15] have been shown to decrease with depth. It was suggested that decreasing C availability caused changes in microbial functions as depth increased [9, 12] and that C availability was a major factor in determining microbial community composition [8]. This pattern is likely to be particularly strong in the scrub-oak ecosystem, where the soil is low in organic matter.

Rhizodeposition, described as organic inputs of living roots into the rhizosphere [18], has potential to change the nutrient conditions of the surrounding soil by increasing P availability [7]. Previous studies in the scrub-oak soil have shown a distinct rhizosphere effect of greater soluble C and N contained in amino compounds and ammonia, greater microbial activity, and more abundant microbial DNA in the rhizosphere compared to the bulk soil [23]. Because of these differences, a larger divergence was expected between the responses of the rhizosphere and bulk soil microbial community.

The objective of this study was to evaluate the feasibility of using the BDOBS to explore nutrient limitation and substrate acclimation across a range of habitats (litter, rhizosphere, bulk soil) in the Florida scrub-oak system. We hypothesized that comparative analysis of the three microbial communities would show that the litter microbial community was the most active due to high microbial densities found in scrub-oak litter compared to the soil (Albarracin, V., Master's Thesis, University of Central Florida, 2005), followed by the rhizosphere microbial community, and the bulk soil community. We examined our hypotheses by comparing the three communities' responses within varied conditions. We also hypothesized that the litter microbial community would be better adapted to litter derived substrates, while soil communities would be better adapted to root-derived substrates and tested this by comparing oxygen consumption of the communities using the two types of natural substrates. It was expected that all communities would be N- and P-limited, but the rhizosphere microbial community would be less P-limited due to potential rhizodeposition-induced soil phosphorus release [7]. In this case, we hypothesized that the addition of N and P would stimulate response, except when P was added to the rhizosphere community. This study provided important validation of the method, particularly since it is the first experiment of this kind using naturally derived substrates and showing the interactive effects of N and P amendment.

Methods

BDOBS

The BD Oxygen Biosensor System is a recently developed assay that utilizes a fluorescing ruthenium dye suspended in a gel in each well of a microtiter plate (BD Oxygen Biosensor system; BD Biosciences, Bedford, MA, USA) [11]. The dye fluoresces as O_2 is depleted in a microbial–substrate–nutrient solution. This fluorescence was measured at set time increments on a Dynex MFX Microplate Fluorometer.

Study Site

The data presented in this study are derived from samples from ambient CO_2 chambers from an open-top chamber, elevated CO_2 experiment established in a Florida scrub-oak ecosystem in 1996. The plant community is comprised of 76% *Quercus myrtifolia*, 15% *Quercus geminata*, 7% *Quercus chapmannii*, *Serenoa repens*, and *Lyonia ferreginea*. The scrub-oak was control-burned in 1986 and again in February of 1996 prior to the construction of the chambers. The experimental site is located on Merritt Island at Kennedy Space Center on the eastern coast of Florida, USA (28°38′ N–80°42′ W at an elevation of 0–3 m above sea level).

There are two soil types on the site, Paolo sand and Pomello sand (see Schmalzer and Hinkle [21] for detailed descriptions). The sandy soils of this system facilitate separation of the three microbial communities of interest (litter, rhizosphere, and bulk soil). The low pH, 3.8 to 4 in the top 15-cm and 4.3 in the 16 to 30-cm depth range [22], may limit nutrient availability in the system. Possible limitations at pH below 4 are N, P, S, B, C, Mg, Fe, Ca, and Mo [25]. The low pH of the scrub-oak soil suggests that acidic pH levels would yield optimal response; preliminary testing was performed to verify this effect.

Preliminary pH Study

In BDOBS, three components were typically added to each microtiter well: microbial inoculum, carbon substrate, and a buffer/nutrient solution. The buffer/nutrient solution was typically buffered at a neutral pH of 7 in the past using BDOBS, but the pH of the scrub-oak ecosystem soil is low (3.75–4). Preliminary pH tests were done to determine the optimal pH for microbial response in this system. A carbonate buffer solution ($KHCO_3$, 10 g/L) was used to adjust the pH. An O horizon, scrub-oak litter community was exposed to various substrates in the two buffered (pH of 5.1 and 7.1) and unbuffered (pH~5) nutrient solutions.

Preliminary Natural Substrate Study

Extracts of fresh and dried samples of roots, litter, and soil were compared. To prepare the substrate extracts, the surface leaf litter (in various states of decomposition) was separated from the soil layer in three combined soil cores (7-cm diameter by 30-cm deep). The remaining soil sample was sieved using a 1-mm mesh sieve to remove the roots. The leaf litter and roots were either dried at 70°C for 48 h for the dry substrate or used fresh for the fresh substrate. The root and litter samples were mixed with water in a 1:5 ratio. The soil was mixed in a 1:2.5 soil to water ratio to maximize organic content of the solution while maintaining a workable solution. Extracts were heated at 46°C for 2 h and filter-sterilized using a 0.22-μm filter. Dried substrates were chosen for use throughout the study because the drying process seemed to release more energy for microbial use (data not shown). Serial dilutions were made of each substrate and utilized by a litter microbial community inoculum (prepared as described below) in the BDOBS system.

Microbial Community Collection and Preparation

Soil cores (containing bulk soil and rhizosphere samples, to a depth of 30 cm; $n=8$) and leaf litter samples ($n=8$) were collected separately through out the summer of 2004. The soil clinging to the roots from the cores constituted the rhizosphere microbial community. The remaining soil was sieved and constituted the bulk soil microbial community. The soil communities were diluted in a 1:2.5 moist soil to sterile ddH_2O ratio and shaken with sterile glass beads (2 mm) for 2 min. The supernatant was immediately poured off and diluted fivefold for plate inoculation, creating a final dilution of 1 g soil to 12.5 mL water.

Approximately 5 g of leaf litter were gathered from each ambient chamber ($n=8$) and fragmented into pieces 3–4 mm in size. The litter fragments were combined with sterilized ddH_2O in a 5 mL to 1 g ratio and shaken for 4 min in a 50-mL centrifuge tube with 2-mm glass beads. The solution was then diluted to a final concentration of 1 g litter to 25 mL filter-sterilized deionized water for plate inoculation.

Nutrient Supplements

Three microbial communities were exposed to four different nutrient treatments, according to instructions from Atlas [2] and modified as described below. An unbuffered, filter-sterilized mineral salt solution (0.01 g $CaCl_2$/L, 0.005 g $FeSO_4$/L, 0.0025 g $MnSO_4$/L, 0.0025 g $NaMoO_4$/L, 0.1 g $MgSO_4$/L) was added to all wells. Nitrogen (0.5 g $[NH_4]_2SO_4$/L) and phosphorus (0.05 g K_2PO_4/L) were added singly and in combination to give four nutrient treatments (−N−P, +N, +P, +N +P) or two high levels of P and N and two ambient levels of P and N.

Carbon Substrates

Three substrates were used for energy sources for microbial growth, along with a water control to monitor the response to background carbon contained in the environmental sample. The three carbon sources were made from litter extracts, root extracts, and glucose. Root and litter substrates were collected as described in Brown et al. [5] and were prepared as described above (see Preliminary Natural Substrate Study). Glucose substrate was mixed with water in a concentration of 600 mg/L and filter-sterilized.

Plate Loading and Reading

BD-oxy plates were loaded with 50 μL of substrate, 50 μL of nutrient solution, and 50 μL of microbial inoculum resulting in final concentrations of nutrients and substrates 1/3 of the stock solutions reported above. The three microbial communities were exposed to all combinations of nutrients and substrates. The fluorescence of the plates was read on a Dynex MFX Microplate Fluorometer plate reader every 15 min for 48 h at 485-nm excitation and 604-nm wavelengths with the bottom-reading mode.

B-Doxy Data Analysis

The fluorescent value was divided by the value at 1 h to obtain a normalized relative fluorescent unit (NRFU). The NRFUs were plotted against time to obtain an oxygen consumption curve (Fig. 1b–d). Response parameters extracted from the curve included time to first response (point in time when NRFU=1.10), the first peak height, and the time to the first peak [11]. Three sets of data (divided by exposure to energy substrate: glucose, background carbon, and natural substrates) were analyzed using MANOVAs, and for each significant response, variables (time to first response, time to first peak, and first peak height) were analyzed using mixed ANOVAs. Significant two- and three-way interactions were analyzed with the least square means post hoc test using SAS (SAS Institute 1990). The fluorescence response (i.e., area under the curve) was calculated using the trapezoid method as a measure of total oxygen consumption. The area was calculated for the response to glucose and background C with +N +P amendment given the lack of response without nutrient supplementation (see Fig. 1, below). To evaluate potential impacts of glucose supplementation on the enhanced used of background C (i.e., soil priming effect [16]), the glucose peak was subtracted from the total response. The area was then recalculated and compared to the samples exposed to background C alone.

Results

Overall Response Types

There were four types of response exhibited by the microbial communities (Fig. 1). First, no appreciable oxygen consumption was exhibited without substrate or nutrient addition (Fig. 1a). When no carbon substrate was added, but N and P were supplemented, a relatively large response was observed after ~36 h of incubation (Fig. 1c). We attributed this response to nutrient-limited use of background C. A single peak response was observed with supplementation with either litter or root substrates irrespective of N or P addition (Fig. 1b). The last type of response, observed with both substrate and nutrient (+N+P) supplementation, consisted of two distinct peaks (Fig. 1d). Based on the other response types, it was concluded that the

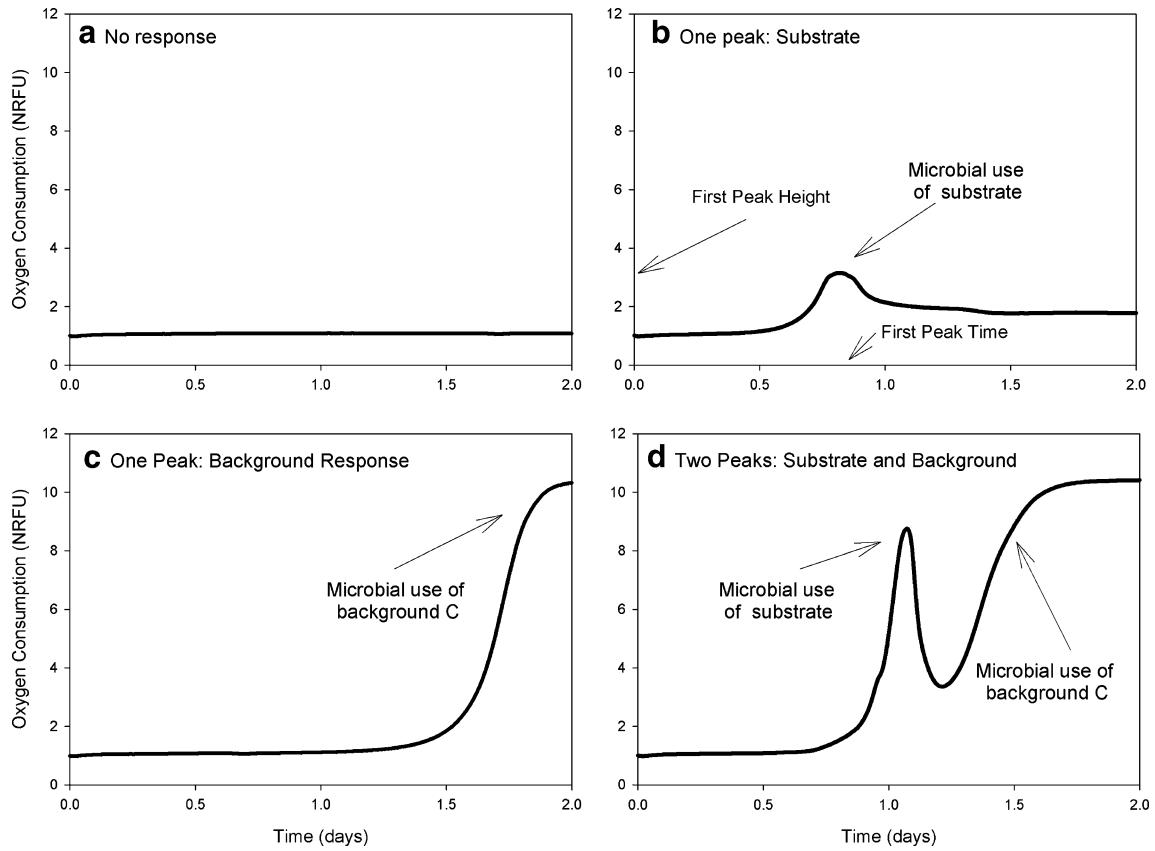

Figure 1 Four types of responses exhibited by the microbial communities. Normalized relative florescence unit (*NRFU*) is the florescence value divided by the value measured at 1 h. **a** No response. **b** One peak: substrate. **c** One peak: background response. **d** Two peaks: substrate and background

first peak was the response to the substrate, and the second peak was the response to the background carbon contained in the environment of the microbial sample (Fig. 1d).

Preliminary pH Study

The litter microbial community responded faster under ambient pH environment (4.75–5 at the study site) than under the two buffered pH environments (pH 5 and pH 7; Fig. 2). The decrease in response was slight at pH 5 (i.e., delay in time to peak response of only 1–2 h), but very substantial at pH 7 (i.e., delay in time to peak from ~0.5 to 1 day). Based on these results, all subsequent testing was performed under ambient pH conditions.

Preliminary Substrate Studies

As expected, response to both the litter and root extracts decreased with increasing dilutions with water (Fig. 3). Response to soil extracts were very slight and soil was eliminated as a substrate. The response to the root extract was greater than litter, even showing sustained maximal fluorescence (indicating complete depletion of dissolved oxygen) for nearly 12 h in the most concentrated form (1:2.5 dilution). We selected specific dilutions of the litter (1:10) and root (1:30) substrates for further testing because they yielded less than maximal response, allowing stimulatory effects of nutrient supplementation to be evaluated. The more concentrated extracts produced more complex signals (i.e., secondary peaks), which may be useful for other applications.

Spatial Comparison of Communities

The litter microbial community tended to respond faster and/or to a greater degree than the soil communities (rhizosphere and bulk soil). The litter microbial community had a faster first response time than the rhizosphere and bulk soil communities (n=8) when using glucose (P<0.0001, Table 1, Fig. 4), background carbon (P<0.0001, Table 2), and the natural substrates (root and litter substrates collectively; P<0.004, Fig. 5) as energy sources. When using the background C, the litter microbial community responded approximately 0.69 to 0.73 days faster than the soil

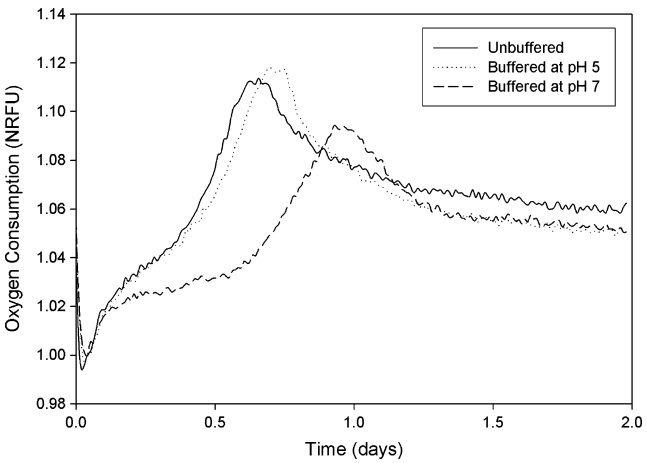

Figure 2 Example of typical litter microbial community response to soil extract in different pH environments. See Fig. 1 caption for explanation of NRFU

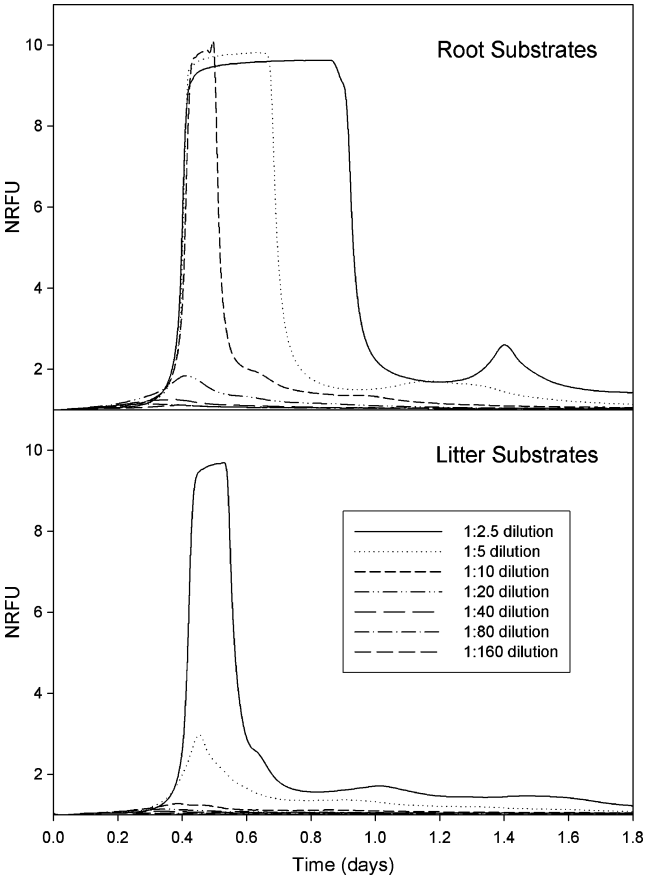

Figure 3 Example of a litter community response to a dilution range of root and litter extracts. See Fig. 1 caption for explanation of NRFU

Table 1 P values for ANOVA results of microbial communities using glucose as an energy source

Variable	1st response	1st peak time	1st peak height
Community	<0.0001	0.0001	0.1309
N	<0.0001	<0.0001	<0.0001
P	0.0368	0.2309	<0.0001
Community×N	0.0181	0.2479	0.3859
Community×P	0.3146	0.6451	0.025
N×P	0.7242	0.0002	<0.0001
Community×N×P	0.2554	0.0456	0.0801

α=0.05, n=8
Community litter, rhizosphere, and bulk soil communities, *N* nitrogen, *P* phosphorus

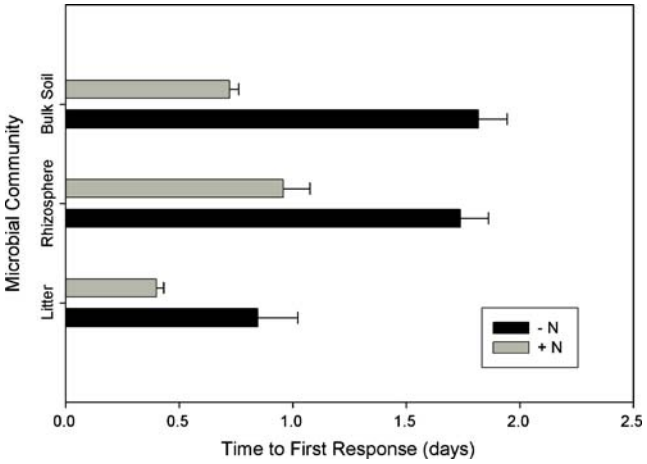

Figure 4 Effects of N and community habitat on time to first response of microbes using glucose as an energy source. The error bars represent one standard error

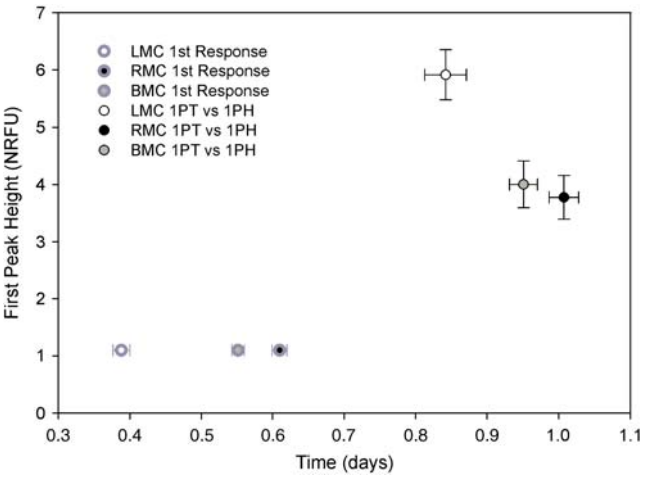

Figure 5 Response of litter microbial community (*LMC*), rhizosphere microbial community (*RMC*), and bulk soil microbial community (*BMC*) to natural substrates (litter and root extracts). *1st Response* represents the time to first response. The height of the first peak (*1PH*) is plotted against the time to the first peak (*1PT*) for each microbial community. The bars represent one standard error for each measurement. See Fig. 1 caption for explanation of NRFU

communities and 0.16 to 0.22 days when using natural substrates. The time to first peak height of the litter communities was faster for natural substrates, but similar for glucose and background C. An exception to this trend was under ambient N, but high P, where the litter community use of background C was significantly faster than the soil communities. Overall, the litter microbial communities did not show a greater degree of oxygen consumption in the form of an increased peak height with the following exceptions. The litter community use of glucose under ambient P showed a significantly higher first peak than the rhizosphere microbial community (but not the bulk soil community, which fell in between), but this difference was not present at high P levels. Also, with litter substrate under high N, the litter microbial community had a significantly higher first peak than both soil communities (Fig. 6b). Overall, the response of the different communities were comparable under any condition, but when differences were apparent, they were related to faster or greater activity in the litter community relative to the rhizosphere and bulk soil communities.

While the bulk soil community appeared to be more active than that rhizosphere community, there was only one instance where this difference was significant. The bulk soil microbial community had a significantly faster time to first response to the natural substrates than the rhizosphere microbial community (Fig. 5).

Table 2 *P* values for ANOVA results of microbial communities using background C as an energy source

Variable	1st response	1st peak time	1st peak height
Community	<0.0001	0.0135	0.4012
N	<0.0001	<0.0001	<0.0001
P	0.4416	0.8388	<0.0001
Community×N	0.1415	0.0983	0.4267
Community×P	0.8946	0.5999	0.2578
N×P	0.1959	0.3064	<0.0001
Community×N×P	0.3121	0.0182	0.2535

α=0.05, n=8
Community litter, rhizosphere, and bulk soil communities, *N* nitrogen, *P* phosphorus

Comparison of Natural Substrates

All microbial communities responded significantly faster to root extract (0.50 days) than litter extract (0.53 days; P=0.0037). Root vs. litter substrate did not seem to affect the time to reach the first peak, but did affect the height of the first peak. There was no significant change of oxygen consumption under ambient N (Fig. 6a), but under high N levels, there was a significant difference (P=0.0115), with each community showing a greater peak response toward root substrates than litter substrates (Fig. 6b). These data indicate that the root substrates contain more labile organic matter, but the use of these substrates is limited by available N (Table 3).

Acclimation to Native Substrates

Litter communities consumed more oxygen when using litter substrates than soil communities (Fig. 6), but this effect was only significant (P<0.001) under high N (Fig. 6b). All communities used root substrates equally well.

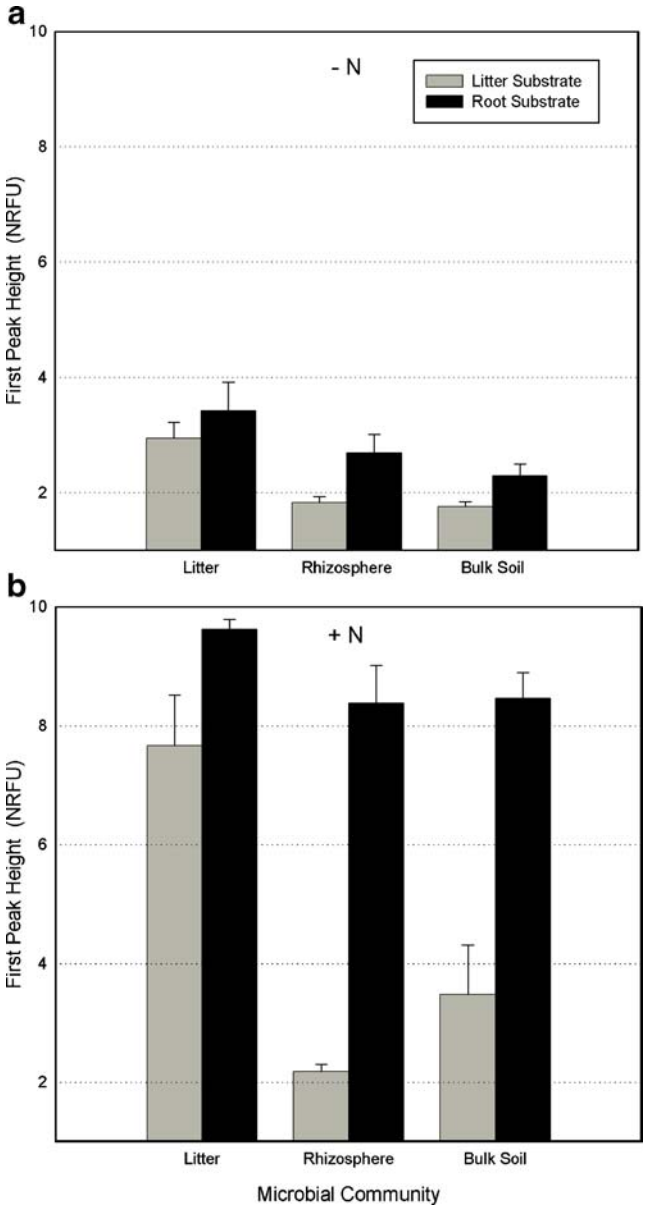

Figure 6 Effect of N (**a** ambient N, **b** high N), P, and community habitat on first peak height of microbes using natural substrates as an energy source. The error bars represent one standard error. See Fig. 1 caption for explanation of NRFU

Nitrogen and Phosphorus Effects

The addition of N tended to enable more extensive use of the natural substrates. N supplementation significantly increased the first peak height (P=0.0115) for root substrate use by all microbial communities. For litter substrates, the stimulatory effect of N was only observed with the litter community (Fig. 6). N addition had no effect on the first response time of the natural substrates (P=0.7844, Fig. 7).

Nitrogen amendment sped up the response of all the microbial communities to background carbon and glucose

Table 3 P values for ANOVA results of microbial communities using natural substrates as an energy source

Variable	1st response	1st peak time	1st peak height
Community	<0.0001	<0.0001	<0.0001
N	0.7844	<0.0001	<0.0001
P	0.5962	0.5162	0.9463
SS	0.0037	0.4413	<0.0001
Community×N	0.9345	0.539	0.0014
Community×P	0.2099	0.8165	0.456
Community×SS	0.9117	0.347	0.0024
N×P	0.3745	0.8181	0.935
N×SS	0.8777	0.4365	<0.0001
P×SS	0.8642	0.8496	0.8591
Community×N×P	0.6403	0.2942	0.4505
Community×N×SS	0.8751	0.7455	0.0115
Community×P×SS	0.5155	0.8214	0.7619
N×P×SS	0.7196	0.6028	0.8915
Community×N×P×SS	0.9759	0.8954	0.7653

α=0.05, n=8
Community litter, rhizosphere, and bulk soil communities, *N* nitrogen, *P* phosphorus, *SS* substrate source (i.e., root or litter extract)

(Fig. 8), but the magnitude of oxygen use was conditional upon P amendment (Fig. 7). Nitrogen addition causes a significantly faster time to first response (P<0.0001) in microbial communities using glucose or only background C (Fig. 8). Phosphorus addition alone sped up the time to first response to glucose for all the microbial communities (P=0.0368), but did not affect the use of background C. Unlike the use of natural substrates, the addition of N alone was not sufficient to cause an increase in overall

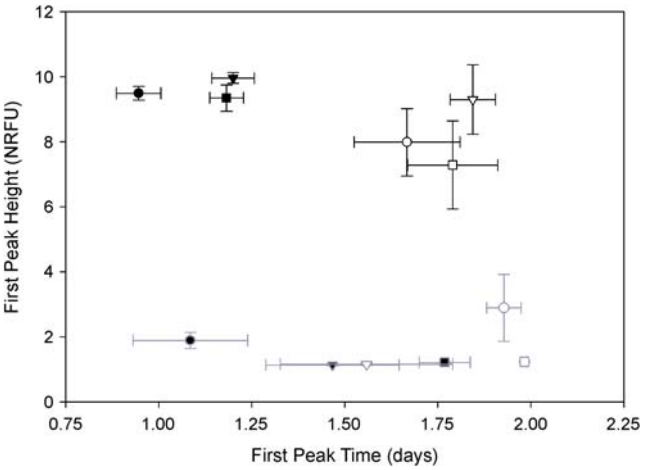

Figure 7 Litter (*circle*), rhizosphere (*triangle*), and bulk soil (*square*) microbial communities' response (first peak time vs. height) to high N while using glucose (*filled symbols*) or background C (*open symbols*) and exposed to high P (*black outline*) and ambient P (*gray outline*). The error bars represent one standard error. See Fig. 1 caption for explanation of NRFU

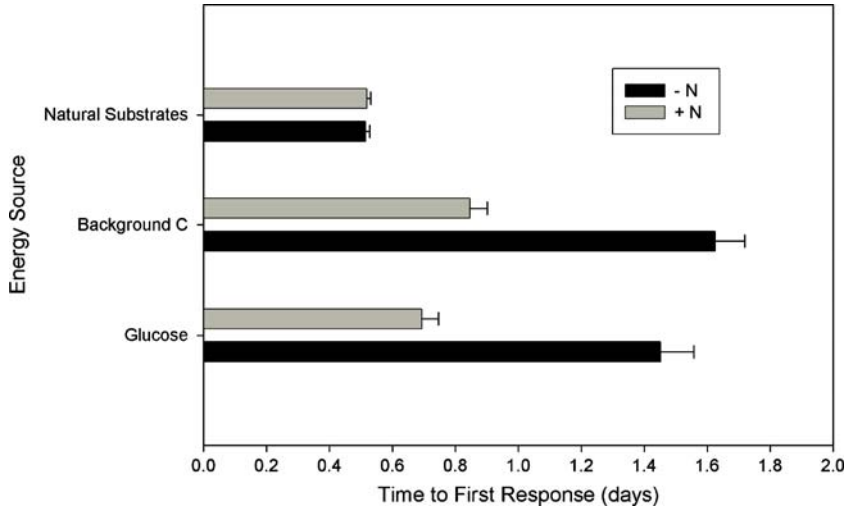

Figure 8 Effect of N addition on the time to first response of microbes using natural substrates, background C, and glucose as energy sources. The error bars represent one standard error

oxygen consumption; addition of P was also required (Fig. 7). In order to quantify this effect, responses were scored on whether or not the microbial community responded to the background C (second peak in the case of glucose) with a NRFU of ≥8 (Fig. 1d), and the percentage of times this occurred for specific treatment combinations was calculated (Table 4). Microbial communities had extensive responses to N addition alone with the use of natural substrates. Addition of both N and P allowed the microbial communities to respond to background carbon when amended with glucose or with background C alone. A response to background C was achieved in only one sample without N addition.

Priming Effect of Glucose

The integral of fluorescence response was greater in glucose amended vs. unamended samples after the area of the glucose peak was subtracted (Fig. 9). These results indicate that the glucose addition caused increased use of the background C for all three communities.

Table 4 Percentage of microbial community response to background C where the peak was greater than eight NRFU

Microbial community	Nutrients	Litter substrate	Root substrate	Glucose	Background C
Litter	−N−P	0	0	0	0
	+N	100	100	0	12.5
	+P	0	0	0	0
	+N+P	100	100	100	75
Rhizosphere	−N−P	0	0	0	0
	+N	100	93	0	0
	+P	0	0	0	0
	+N+P	100	100	100	87.5
Bulk soil	−N−P	0	0	0	0
	+N	100	93	0	0
	+P	0	0	0	0
	+N+P	93	100	100	62.5

n=8

Discussion

Spatial Comparison of Communities

The predicted ranking of microbial communities by activity (litter > rhizosphere > bulk soil) was partially substantiated by the data. The litter responded faster and consumed more oxygen than the other communities, confirming conclusions of previous studies with the Florida Scrub-oak (Albarracin, V., Master's Thesis, University of Central Florida, 2005). The unexpected response of the scrub-oak communities was that the bulk soil community was typically more responsive than the rhizosphere microbial community, although the differences were not always significant. The rhizosphere was expected to be more active due to the stimulation from root exudates [19], but our results concur with work from Butler et al. [6] and Steer and Harris [24] who found little difference in PLFA profiles and microbial biomass between the rhizosphere and bulk soil microbes.. Another possible explanation for the unexpectedly lower rhizosphere response may be related to disturbance associated with the sampling method. The rhizosphere is a gradient of compounds, nutrients, and root exudates, varying in concentration from the root surface to the surrounding soil. The microbes may be specifically adapted to their position within the gradient. Disturbance to these microenvironments may have delayed recovery time. The bulk soil is a homogeneous environment in comparison to the rhizosphere, and disturbance may not have as adversely affected the microbes.

Comparison of Natural Substrates

Response to root substrate was greater than the response to litter substrate for all communities, including the litter community. This trend was observed despite the higher measured caloric content of the leaf litter compared to live roots [4] used for the extractions. The greater response to root substrates is most likely due to the use of live roots compared to dead litter, resulting in a greater proportion of labile organic matter in the extract from roots.

Acclimation to Native Substrates

The relative recalcitrance of the litter substrate may have allowed for detection of substrate acclimation in its use. The litter microbial community respired a larger fraction of the soluble organic carbon of the litter extract than the soil communities. The lower response of the soil microbial communities suggests a lack of enzymatic potential, either due to differences in community composition or physiological status, toward some components of the leaf litter extract. The ability to detect acclimation to local resources amongst spatially distinct microbial communities indicates that natural substrates are a useful addition to the suite of compounds used for CLPP.

Nitrogen and Phosphorus Effects

The BDOBS system clearly demonstrated primary N and secondary P limitation of microbial activity in all the habitats. The degree of nutrient limitation in these soils appears particularly severe as indicated by the complete lack of response to glucose amendment without the addition of N and P. Other studies employing various methods [1, 14, 27], including some using the CLPP-BDOBS approach [28], reported increased activity and/or biomass upon the addition of labile carbon to other types of soil without nutrient supplementation. Many studies have revealed increased microbial respiration in response to N addition, often linked to C availability in the soil. The addition of N alone to the soil in Allen and Schlesinger's study [1] stimulated microbial respiration, but in the scrub-oak ecosystem, either P or a natural substrate was needed in conjunction with N to elicit a response. The natural substrate may contain enough P to prevent limitation. The ability of the litter community to use some substrate when exposed to N+ background C or N+ glucose indicated that the litter community has relatively more P available than the soil communities. Vance and Chapin [27] found that the response to N addition was greatest when there was abundant C availability. Both Vance and Chapin [27] and Allen and Schlesinger [1] found a significant interaction of C and N addition, but neither required the addition of P for stimulation of microbial activity. Phosphorus addition significantly decreased the time to first response for microbial communities utilizing glucose as an energy source. This effect could be related to an increased ability to generate ATP and process glucose for cell maintenance, but may not necessarily lead to an increase in cellular biomass or cell replication, unless N limitation is also removed.

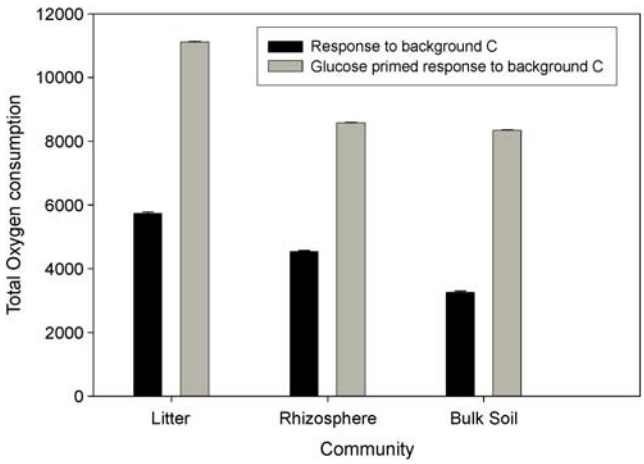

Figure 9 Response of microbial communities to background C only with N and P addition: a comparison of communities primed by glucose to those not primed by glucose. The error bars represent one standard error

The C available in the soil, as indicated by the secondary peak in respiration when N and P limitation are removed, was surprisingly large for what is considered a carbon poor soil. The fluorescent signal was at maximal level for at least several hours, a response typically observed when >500 mg L^{-1} of labile carbon is added to soil [10]. While further study is needed to characterize the carbon fueling the secondary peak, the presence of this large response suggests that a large pool of labile carbon exists in these soils. The causes for the accumulation of this reservoir may be related to the severe nutrient limitation of the soils and/or the strong wetting and drying cycles, which occur in these sandy soils exposed to heavy, intermittent rainfall.

Priming Effect of Glucose

In many past studies, the priming effect was measured using evolution of CO_2, NH_4, or NH_3 [16], whereas we measured the priming effect with O_2 consumption. By subtracting the response to the C amendment (i.e., the clearly discernable first peak), we demonstrated that there was a priming effect, but that N, P, and glucose were the necessary triggers for the communities. Many studies of the priming effect have used manure as a trigger and, therefore, may have involved a combination of these factors (C, N, and P) in the observed priming effect. To our knowledge,

this is the only study that explicitly demonstrated that N, P, and C were all required to trigger a priming effect [16]. Because the oxygen consumption was only measured for 48 h, we do not know how long the plateau would have extended and, therefore, cannot say that there was an overall increase in O_2 consumption (use of background C) with the addition of glucose. We can state that the addition of glucose, N, and P caused a more rapid stimulation (i.e., within 48 h) in the use of background C. These results indicate that the BDOBS-CLPP approach may be a useful approach to rapidly evaluate the role of multiple factors on the priming effect in soil.

Versatility of BDOBS

In addition to the advantages outlined above, the versatility of the system will allow for its application in almost any situation where microbial oxygen use is of interest. For example, it could potentially be used to observe effects of various allelopathic chemicals on soil microbes that form mutualistic relationships with plants. It could also be used in wetland systems to examine the variation of response to oxygen exposure along the gradient of oxygen availability from the rhizoplane outward. Because of the increased sensitivity of the system, it is feasible to use inoculums from sources that in previous methods have not yielded sufficient material, such as insect guts, the rhizosphere, or soil aggregates. In the first study of soil systems using this technique, Väisänen et al. [26] used BDOBS to successfully isolate fungal and bacterial responses and compare microbial community response by aggregate sizes. In the Florida scrub-oak, we examined the litter community, the rhizosphere, and the bulk soil communities' responses, which would not have been feasible on other systems due to either the opacity of the soil slurry or the minute quantities of rhizosphere soils collected.

Conclusions

Use of the BDOBS facilitated observation of the different responses of the three microbial communities. The litter community was more responsive than the soil communities, probably due to higher abundance of microbes typically found in the litter layer. Differentiation between the responses of the two soil communities (rhizosphere vs. bulk soil) was less pronounced than expected, but still present. Finally, there was evidence of the acclimation of litter communities to use of litter extracts under high N conditions when compared to soil communities. The oxygen biosensor system also allowed observation of increased microbial community oxygen consumption with nutrient additions and the observation of a priming effect. The system was N- and P-limited, preventing the use of C available in this system, unlike other systems where C is the limiting factor for microbial communities.

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