Author

Devan Nisson began her project as a way to develop both her advanced laboratory skills and computational experience in the field of microbial ecology while conducting research independently. Beyond the research presented here, she plans to expand her project to investigate various other means of microbial metabolic response to drought. Devan particularly enjoyed the opportunity to design the experimental setup for her project, and feels that the critical thinking skills and exposure to new experimental techniques she gained from this experience are invaluable to her growth as a biological researcher. After graduation, Devan intends to continue her studies, preparing for a career as a biological researcher.

Key Terms

- Biome
- Birch Effect
- Climate Change
- Desert
- Drought
- Microbial Community
- Respiration

Adaptive Metabolic Response of Desert Microorganisms to Drought and Moisture Pulses

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Abstract

Soil microbial communities metabolize organic matter in dead plant material by enzymatically degrading macromolecule components. The total respiration of carbon dioxide represents an integrated metric of this degradation process. This study compares the metabolic performance of microbial communities from ecosystems arrayed along a climate gradient, including desert, scrubland, grassland, pine-oak forest, and subalpine forest. Specifically, we test whether or not desert microbes display adaptations for increased metabolism relative to the other communities upon rewetting after extended periods of drought and high temperature exposure. To simulate desert conditions, we exposed microbial communities from along the gradient to moisture pulses after three, six, or nine weeks of incubation at 31 ºC on grassland leaf litter. Carbon dioxide readings were recorded every two days. We found that each type of community, regardless of its original biome, displayed both susceptibility and resilience to drought-like conditions, each exhibiting a gradual recovery to pre-treatment respiration rates. This shows that litter-inhabiting microbial communities are capable of functioning under environmental conditions vastly different from those of their original ecosystems. These results suggest that diverse microbial communities may be able to withstand drought conditions caused by climate change events.

Faculty Mentor

The climate is getting hotter, and in places like Southern California, it's also getting drier. Devan's study tests how microbes like bacteria and fungi will cope. Microbes promote soil fertility and recycle nutrients, helping to maintain plant growth in natural and agriculture systems alike. After subjecting Southern California microbes to hot, dry conditions in the laboratory, Devan found that microbial communities handled stressful conditions surprisingly well, even if they came from cool, wet mountaintop environments. It is possible that the naturally variable climate in Southern California—where it can get hot even in the mountains—may prime microbes to deal with climate change. This insight would not have been possible without Devan's creative experimental design as an undergraduate researcher.

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School of Biological Sciences

Introduction

Large reservoirs of organic carbon are present in the soils of most terrestrial ecosystems (Todd-Brown et al., 2013). The carbon harbored in these soils is released largely by the metabolic activities of native microorganism communities. These microbial communities, primarily composed of bacterial and fungal species, degrade a variety of carbon-containing macromolecules present in leaf-litter substrates, respiring carbon dioxide into the atmosphere as a byproduct (Vetter et al., 1998; Bardgett, 2005) (Figure 1).

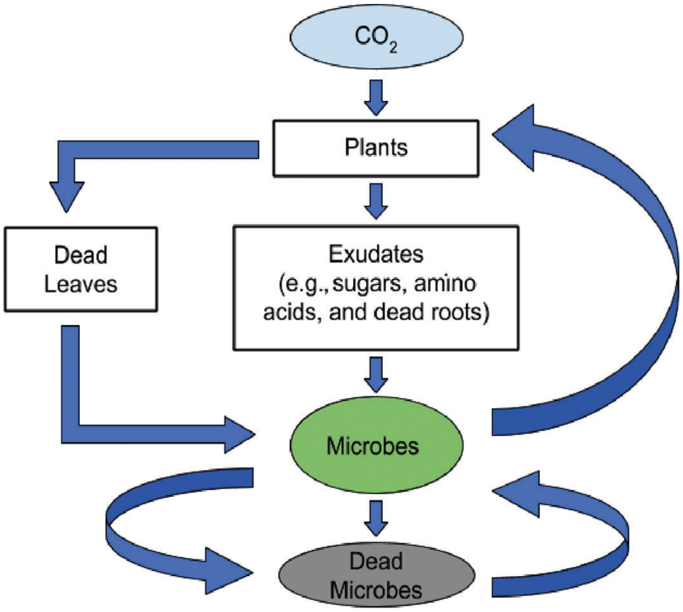

Figure 1

Microbial role in the terrestrial carbon cycle: soil-dwelling microbial communities degrade organic matter in the form of leaf-litter substrates including dead leaves, root exudates (*e.g.*, sugars and amino acids), and dead roots. Other dead microbes can also serve as a source of organic carbon for microbial degradation. These microbes release carbon dioxide back into the atmosphere as a metabolic byproduct.

The abiotic and biotic constraints characteristic to each distinct environment, or ecosystem, may lead to differences in microbial community composition and metabolic activity (Allison et al., 2013). Because these microorganisms are among the primary decomposers of soil organic carbon, it is essential to understand not only how their degradative potential is influenced by their native environmental conditions, but also how their metabolic capabilities may be affected by changes in climate (Swift et al., 1979). This is an especially pressing question, as many ecosystems across the globe, including those in southern California, face the imminent threat of warming temperatures and drought as a consequence of climate change (Seager et al., 2007). Previous research has considered the effect of increased temperatures on microbial respiration in annually moist biomes, such as peatland, boreal systems, and wetlands. Such studies suggest that microbial communities in these systems display an overall positive respiratory response to climate warming, with some initial sensitivity to temperature increase (Allison and Treseder 2008; Davidson and Janssens, 2006). However, while the positive response of moisture-dependent communities is interesting alone, these communities may be outperformed substantially by communities from naturally arid environments upon exposure to long periods of drying as a consequence of warming. To further our understanding of microbial metabolic response to drought, it is necessary to not only consider the respiration of communities from wetter, cooler ecosystems, but also the response of communities normally exposed to low- moisture, high-temperature conditions.

In order to analyze these differences, we developed a laboratory microcosm experiment to test whether a desert microbial community would display an adaptive metabolic response to moisture pulses following warming-induced dry conditions when compared to communities from wetter, cooler ecosystems. This metabolic response can be quantified by measuring the rate of respiration (*i.e.* rate of CO_2 production). This study also aims to test for a "Birch Effect" in the response of each community to moisture pulses after prolonged periods of drying. The Birch Effect hypothesis is that communities, regardless of biome origin, will display increased respiration in proportion to the length of the dry period preceding a moisture pulse. As drying continues, some community members will die off and serve as additional substrate for surviving microbes; this accumulation of substrate will allow for greater spikes of respiration following a moisture pulse (Jarvis 2007) (Figure 2).

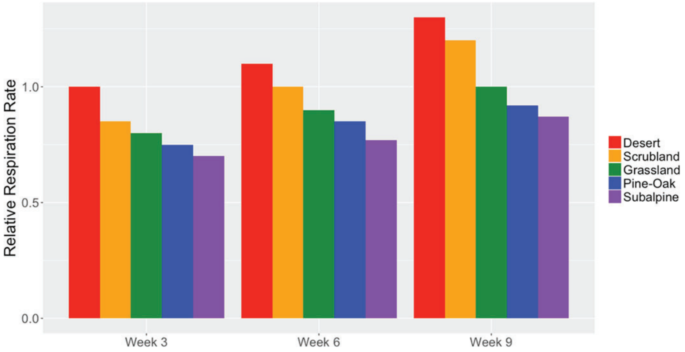

Figure 2

Hypothetical CO_2 production after rewetting relative to initial production for communities from each ecosystem type at each time point, displaying the Birch Effect.

We tested the following specific hypotheses using our microcosm design:

(1) The desert microbial community should display an adaptive response to drought and high temperature, resulting in a greater respiration rate upon each exposure to a moisture pulse when compared to the other communities.

(2) All communities should display increased respiration upon exposure to a moisture pulse after prolonged periods of drying, as a consequence of high temperature incubation—thus exhibiting a "Birch Effect."

Materials and Methods

Site Description

Samples of litter microbial communities were collected on October 23 and 24, 2016, from five ecosystems along a climate gradient in southern California, spanning 20 °C and 500 mm precipitation, including: desert, scrubland, grassland, pine-oak forest, and subalpine forest (Baker and Allison, 2017) (Table 1).

An incubation temperature of 31 °C was used throughout the experiment, obtained from averaging monthly low and high temperature values for the three hottest months of the year: July, August, and September. These values were provided from the Boyd Deep Canyon Desert Research Center located on the western edge of the Colorado Desert, eight kilometers south of Palm Desert, California.

Microcosm Design

In order to measure the respiration rate from each of the five communities under desert-like conditions, we inoculated each community onto a common litter substrate in pre-sterilized 40 ml vials (microcosms). Community samples were collected from standing litter outside each of four experimental plots maintained by UC Irvine at each site. These samples were ground to fragment lengths of <0.5cm (Baker and Allison, 2017). From each plot sample, 1.0g was combined to establish a representative litter community inoculum sample for each site. Individually, 0.1g of each inoculum type was introduced to vials containing 1.0g of sterile, ground (<2.0cm fragments) grassland leaf litter substrate.

Our design included three replicate sets of 36 vials (Figure 3). Each vial only harbored one community at any given time. The grassland substrate was both gamma-irradiated and autoclaved to ensure sterility. The use of one common, sterile substrate in all the vials was necessary to eliminate bias from selective degradation of different leaf-litter substrates by the various communities; grassland substrate was selected because grass was present in all of the ecosystems along the gradient. Immediately after inoculation, 5.6 mL of ultrapure water was introduced to all vials to allow the communities to establish on the substrate. All vials designated "week 1" were rewetted (received a moisture pulse) one week post-inoculation and were used for initial respiration rate calculations throughout this study.

In each set, three additional time points of moisture introduction were used to evaluate community respiration after different lengths of incubation at high temperature, including: week 3, week 6, and week 9. Vials were only rewetted once and then destructively harvested. One moisture-receiving vial (the vials depicted with thick black borders in Figure 3) for each community was assigned to each of the

Table 1

Site descriptions for microbial community sample collection. Ecosystems were chosen to represent a gradient of moisture and temperature across southern California.

Biome Type	**Site Location**	**Latitude, Longitude**	**Average Annual Precipitation (mm)**	**Average Annual Temperature (°C)**
Desert	Philip L. Boyd Deep Canyon Desert Research Center	33.65N, 116.37W	129	24.5
Scrubland	Burns Piñon Ridge Reserve	33.61N, 116.46W	313	18.0
Grassland	Loma Ridge— Limestone Canyon	33.74N, 117.70W	408	18.7
Pine-Oak	James San Jacinto Mountains Reserve	33.81N, 116.77W	574	12.5
Subalpine	San Jacinto Mountains Subalpine Forest	33.80N, 116.69W	648	4.3

three time points of wetting. Moisture-receiving vials designated at either the week 3, week 6, or week 9 time point were paired with a control vial in which no moisture was introduced. The week 1 vials were not paired, since these vials served only as an initial indicator of microbial metabolic activity, and all received moisture. Additionally, a control vial was included that contained only sterile grassland litter and no microbial community.

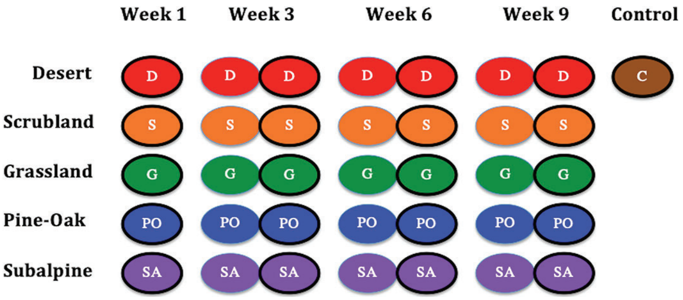

Figure 3

Microcosm setup. One set of 36 vials is shown (3 sets, 108 vials in total). Vials rewetted at week 1 were used to determine initial respiration rates for each community. Vials designated week 3, week 6, and week 9 represent the time of rewetting after inoculation.

CO_2 Measurements

Rewetting, either at week 1 or subsequent weeks, consisted of adding 5.6 mL of ultrapure water in order to moisten the litter to field capacity. After water addition, vials were capped and placed back in the incubator at 31 ºC for 5.5 hours. After this period, 10 mL of air was extracted from each vial using a sterile syringe; this air was then injected into an EGM-4 infrared gas analyzer for quantification of CO_2 in parts per million (ppm) (Allison et al., 2009). Initial CO_2 production was compared across the five communities to determine if there were any differences in community metabolism one week after inoculation. This initial measurement was taken to be the respiratory response of vials rewetted at week 1. One week 9 vial for the desert community was excluded due to a break in the rubber cap over the accumulation period.

Calculations and Statistical Analysis

CO_2 concentrations were converted from parts per million (ppm) to micromoles per gram per hour ($\frac{\mu mol}{g \cdot hr}$), providing a rate of microbial substrate degradation for each community. This conversion was calculated with equation 1, adapted from Dossa *et al.*, 2015:

$$R_{CO_2} = \frac{CO_2}{dt} \cdot \frac{P(V_v)}{RT} \cdot \frac{1}{W_s} \tag{1}$$

Where R_{CO2} is the rate of CO_2 respired in ($\mu mol \cdot g^{-1} \cdot hr^{-1}$), $\frac{dCO2}{dt}$ is the change in carbon dioxide concentration (ppm) over time (where ppm is equivalent to $\frac{\mu mol}{mol}$), P is assumed atmospheric pressure of 1 atm, V_v represents the volume of the vial, R is the gas constant in $\frac{mL \cdot atm}{\mu mol \cdot K}$, T is incubation temperature in Kelvin, and W_s is the dry weight of the sample in grams.

A relative index of CO_2 production for each time point of water addition (week 3, week 6, and week 9) was calculated by dividing the average respiration rate for vials wet at that time point by the average initial respiration rate from vials wet at week 1. This calculation, equation 2, was performed for each ecosystem:

$$Relative\ Respiration\ Rate = \frac{Average\ CO_2\ Respiration\ Rate\ (Post\ Moisture)}{Average\ Initial\ Respiration\ Rate} \tag{2}$$

Statistical significance of the community effect on respiration was evaluated for initial respiration rates by using a one-way ANOVA test on raw respiration values.

Additionally, statistical significance was tested by considering the independent factors of time and ecosystem type at each rewetting. For this evaluation, a two-way ANOVA test was applied across relative index values, in addition to a separate test applied to raw respiration values, from the five communities. A significance cutoff value of $\alpha = 0.05$ was considered for all analyses. If the two-way ANOVA revealed statistical significance, then a Tukey Post Hoc comparison test was performed to evaluate specific differences.

Results

Comparison of Initial Respiration Rates

There was a significant difference in initial respiration rates as determined by the one-way ANOVA analysis ($p = 0.023$), with the Tukey analysis revealing a difference between grassland and subalpine respiration values ($p = 0.024$). All other community comparisons displayed similar initial metabolism after inoculation and wetting (Figure 4).

CO_2 Production Post-Moisture

The two-way ANOVA analysis for relative respiration values, using time and community type as independent factors, did not yield any statistically significant differences between the desert community and the other communities at each time point of wetting. Furthermore, considering each community's metabolic performance across the three time points of wetting, no one community type displayed a

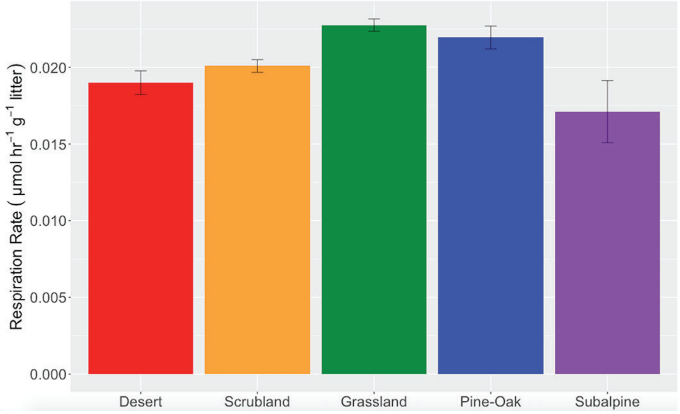

Figure 4

Average initial rates of CO_2 production for each of the five ecosystems (week 1). Error bars represent mean ± SE (n=3).

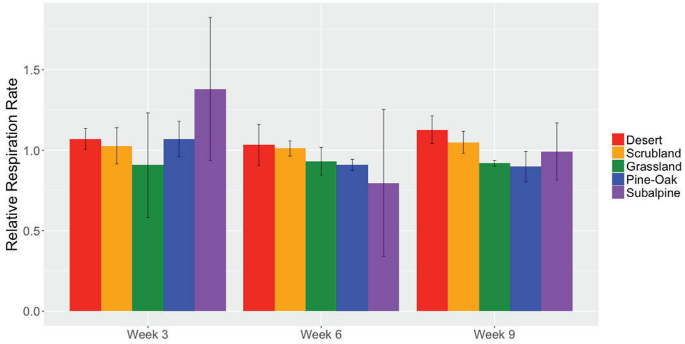

Figure 5

Relative comparison of respiration rates after rewetting to initial respiration rates for communities from each biome type at each time point. Error bars represent ± SD of relative respiration for re-wetted vials (n=3).

significant difference in its respiration rate over time ($p = 0.563$) (Figure 5).

Considering raw respiration values, there was a statistically significant decrease in overall respiration rates (across all communities) between week 3 and week 6 ($p = 0.0495$). There was no significant difference detected between overall raw respiration rates in week 6 versus week 9 or week 3 versus week 9 ($p = 0.9275$ and $p = 0.1068$, respectively).

Respiration was compared between control vials and all inoculated vials at week 1 to confirm sterility. The average initial respiration rates for sterile control vials and all non-sterile vials were calculated separately, with standard error, as $0.0003022 \frac{\mu mol}{g \cdot hr}$ (± 0.0001343) and $0.07565 \frac{\mu mol}{g \cdot hr}$ (± 0.0007597), respectively.

Discussion

To the fields of ecology, evolution, and microbiology, understanding the effects of climate change is incredibly important for predicting how ecosystems will adapt and function in the future, including the effects that warmer temperatures will have on microbial communities. Since the effects of elevated temperatures on a global scale are still relatively unknown, it is crucial to study how microbial communities will adapt in response to increasingly arid environments and periods of drought.

The communities used in this study were derived from a climate gradient of ecosystem types, from warmer, drier communities to those in cooler, wetter forests. Incubation at high temperature and exposure to moisture pulses simulated drought-like conditions under which each community's metabolic performance could be examined relative to the desert microbial community. Since the desert community is normally exposed to arid conditions, we predicted that it would metabolically outperform the other communities at each time point of wetting by displaying a greater rate of respiration, according to hypothesis 1.

At the initial time point of wetting (week 1) similar respiration values were found for all communities except for a significant difference in grassland and subalpine community respiration values. Since the subalpine site receives the most moisture annually, this community may not have needed to capitalize on initial moisture introduction. Furthermore, because moisture had been added to every vial at the onset of the experiment to initiate inoculation, all communities were kept relatively moist in the days prior to the week 1 wetting. Generally similar initial respiration values confirmed no one community was establishing incredibly well or poorly on the substrate at the onset of the study.

In contrast to hypothesis 1, the desert microbial community did not outperform the communities from wetter, cooler biomes after incubation at high temperature, according to both two-way ANOVA analyses. This suggests the desert microbes did not have an adaptive advantage to high-temperature, drought-like conditions. However, the results shown in Figure 5 do suggest that the desert microbial community outperformed the other communities at later time points of wetting (week 6 and week 9) upon qualitative comparison. Over long periods of exposure to high temperatures, thermophilic species have been shown to adaptively outcompete their temperate counterparts, and such heat-loving species may be more abundant in the desert community (Pietikäinen et al., 2005). If this study were extended to include even longer durations of drying, the desert microbial community could eventually significantly outperform the wetter, cooler communities (Bradford, 2013; Luo, 2001). A future analysis of the microbial species abundance in each community over long periods of incubation would further aid in the understanding of specific community adaptation to climate warming and drying over time (Wallenstein and Hall, 2011).

Hypothesis 2 proposed greater spikes in CO_2 production for each community with increasing duration of drought, a phenomenon known as the Birch Effect (figure 4). In accordance with this effect, longer dry periods should lead to more cells dying, supplying more substrate available for respiration upon wet-up (Unger et al., 2010). The statistically significant decrease in overall raw respiration rates between week 3 and week 6 suggests that it takes longer than 3 weeks for the communities to dry out and for individual microbes to start dying. Furthermore, since there was no statistically significant increase in overall raw respiration rates between week 6 and week 9, there is a longer delay period between the time it takes for treatment to impact metabolic performance and the time it takes for communities to significantly outperform their initial activity.

Individual comparisons of community performance over time similarly did not show a statistically significant increase. However, an increase in community respiration rate was observed across the desert, scrubland, and subalpine ecosystems from week 6 to week 9, with the pine oak and grassland sites maintaining similar respiration rates. This increase in several communities does suggest some support for the Birch Effect hypothesis. Overall, the communities were either increasing their metabolism of the grassland litter, or the surviving microbes were able to capitalize on degradation of their deceased counterparts (Jarvis 2007).

All microbial communities, independent of their origin, were able to take advantage of sudden moisture pulses and the majority even displayed a gradual return to pre-treatment respiration rates (Figure 5). It has been proposed that microbial communities, regardless of their origin, may be able to acclimate relatively quickly to perturbations in climate, a pattern termed resilience. This resilience may be due to the ability of microbes to gradually adapt or acclimate their physiological metabolism of organic matter, so that respiration over time becomes similar to pre-treatment community respiration (Shade et al., 2012; Allison and Martiny 2008). These results support the potential of microbial communities from a wide variety of ecosystems to metabolically adapt in the face of drought while also elucidating the initial susceptibility of each community to these drought-like conditions. It is important to consider that all of these southern CA communities are likely to experience extended periods of drought and relatively high temperature in the summer period, which spans 4–6 months (Alster et al., 2013). The initial vulnerability and gradual recovery displayed in this study may be the result of common adaptation mechanisms possessed by all communities. In combination with the results from this study, further research to explore the effects of climate change on microbial communities in various ecosystems, over extended periods of drying, would provide even greater insight to the changes in microbial ecology under long-term climate change.

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