Dormancy rates in microbial communities across an elevational gradient

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Independent research and Methods in Field Ecology
Summer 2017

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Abstract

Despite the broad impacts microbial dormancy has on ecosystems and ecosystem models, dormancy as a response to climate-change-related environmental stressors is poorly understood. Dormancy is an essential bet-hedging strategy used by microbes to tolerate unfavorable conditions and increase overall community diversity. Accurate estimates of dormancy improve existing models of microbial systems. However, the ways in which climatic gradients shape the level of dormancy in soil bacterial and fungal communities remains unclear. Here, we performed a genomics-based, integrated field and laboratory study to evaluate the level of dormancy in bacterial and fungal communities across a natural climate gradient to test if dormancy in bacterial and fungal communities was related to changes in soil properties and climatic factors. To test this question, we examined microbial dormancy at five sites along an elevational gradient near Crested Butte, CO. Additionally, temporal changes over the growing season may shift dormancy patterns at a single site, leading us to collect weekly samples from a low and high elevation site to compare dormancy shifts among sites and temporal shifts within sites. Dormancy work is ongoing, however some preliminary evidence has shown that pH decreases from low to high elevation and increases over the growing season. A better grasp of microbial dormancy will improve our ability to model microbial communities and ecosystems, thus leading to a more thorough understanding of the effects climate change will have on microbial communities and the myriad of environmental processes that they support.

Introduction

Microbial communities are ubiquitous in soil, constitute a majority of global biodiversity, and play key roles in the functioning of terrestrial ecosystems (Meng et al. 2013). Microbes support organic matter decomposition, nutrient cycling, and water uptake by vegetation (Panikov et al. 1999) (Hofman et al. 2016) (de Vries et al. 2013). Understanding the distribution and diversity of microbial communities in an ecosystem is key to characterizing that system as a whole. Microbial community structure has been shown to differ along a climate gradient (Whitaker et al. 2014) and on pH and nutrient enrichment gradients (Mueller et al. 2016), but the role dormancy plays in shaping these structures has not been determined.

Dormancy is a survival mechanism common to microbes in many types of systems (Wang et al. 2014). In unfavorable conditions, microbes can enter a reversible dormant state to preserve the genetic code until conditions improve to allow for replication (Wang et al. 2015), which indirectly allows microbial communities to

maintain high levels of biodiversity (Jones et al 2010; Kearns et al. 2016). A thorough understanding of dormancy is necessary to generate accurate models of microbial systems. Dormancy is critical to soil nutrient pools and nutrient fluxes entering and exiting ecosystems; however, most global carbon models do not currently account for dormancy.

A community's level of dormancy can be measured in several ways. The ratio of rRNA to rDNA has been used to estimate the size of the dormant population in a given community; the thought being that active microbes will be growing and growing microbes have increased levels of rRNA. However, this method has been shown to be at times inaccurate or misused (Blazewicz et al. 2013). Another, more precise method of community analysis is to evaluate the relative abundance of a gene associated with transitions from dormant to active states, the resuscitation promoting factor C (*rfpC*), which has been found in numerous metagenomes and is prevalent in soils (Mueller et al. 2016) (Lanzén et al. 2016). A significant positive correlation has been found between the proportion of active OTUs and the relative abundance of *rpfC* genes (Mueller et al. 2016).

Soil mineral resources, solar radiation, amount and quality of biosynthetic products, and mass-transfer factors all shape soil microbial communities (Panikov 1999), and climate change can affect these factors in a plethora of ways. Because microbial communities are structured by a myriad of direct and indirect interactions between soil properties, climate, and aboveground plant community, global change will reshape microbial communities through multiple and simultaneous pathways (Panikov 1999) (Mueller 2016).

Aims

The aim of this study was to characterize and compare bacterial and fungal dormancy as a response to changing climate and soil properties. The null hypothesis stated that climate-related environmental changes would have no effect on the relative dormant and active populations of bacterial and fungal communities across climatic gradients. We predicted that climate would shape the dormancy patterns in bacterial and fungal populations and that the biotic and abiotic factors shaping dormancy would differ between bacteria and fungi.

The objectives of this study were threefold:
Objective 1. Characterize soil properties across the climate gradient.
Objective 2. Determine the relative populations of dormant and active bacteria and fungi across the climate gradient.

Objective 3. Analyze the relationship between bacterial and fungal rates of dormancy and climate-related soil and environmental chemistry changes.

Methods

To address the questions above, we conducted our study along a pre-existing elevational gradient near Crested Butte, CO (Fig 1, Read et al. 2017, Henning et al. in prep). We measured microbial communities at 6 sites that varied in elevation from 2,400 to 3,400 m above sea level, along a sub-transect design (Fig X).

Objective 1. To characterize site-level soil properties and climatic properties at each site, we used a combination of pre-existing plant community, slope and aspect, and soil moisture data, as well as Worldclim databases for climatic data. Additionally, we collected fresh soil to measure pH, conductivity, and soil carbon content. We measured soil pH using an Orion 3 Star portable pH probe (Thermo Scientific, Waltham) on a 20g air-dried soil slurry in deionized water. Next, we measured soil conductivity a conductivity probe (Oakton Instruments, Vernon Hills) on the extract from the same soil slurry.

Objective 2. To determine the relative populations of dormant and active bacteria and fungi across the climate gradient, we collected samples were collected at Jeremiah Henning's observational sites at Almont (2740 m), Elkton (3200 m), Judd Falls (2943 m), Washington Gulch (2806 m), Painter Boy (3392 m) and Cinnamon Mountain(3460 m). At each site, we collected soil samples in a sub-transect design with sampling sites collected at either 0.5m or 1.0m increments along the transect line (Fig X). This allowed us to measure spatial variation within microbial communities and dormancy at multiple scales. Additionally, we sought to determine the relative role of spatial versus temporal variation in microbial dormancy so at a low (Almont) and high (Cinnamon) elevation site, we took repeated samples weekly throughout the growing season. Soil cores were taken by XXX…. and were flash-frozen on dry ice in the field and stored at -80 ℃.

Molecular analysis of microbial dormancy will use similar methodology as Mueller and Hesse (2016). Briefly, we will extract total DNA and RNA from soil using MoBio DNeasy and RNeasy kits (Qiagen, Carlsbad CA, USA). Prior to sequencing, we will convert RNA to complementary DNA (cDNA) by incubating samples with random hexamers and then SMARTscribe Reverse Transcriptase Kit (Clontech, Mountain View CA, USA). Next-generation shotgun sequencing will be performed on processed samples.

The abundance of active vs. dormant operational taxonomic units (OTUs) in the bacterial and fungal communities will be determined via the relative recovery of sequences from rRNA and rDNA (Jones et al. 2010). Bacterial dormancy levels will

be further confirmed by the relative presence of the gene *rpfC*, which is associated with resuscitation from dormancy and has been shown to correlate strongly with relative abundance of active microbes (Mueller et al. 2016).
Objective 3. To analyse the role of climatic and edaphic factors on determining the rate of bacterial and fungal rates of dormancy we will perform multiple regression and Canonical correspondence analysis (CCA) on seasonal and gradient samples. This will allow us to determine the drivers of dormancy within microbial communities.

Results

Overall, we have found that edaphic and climatic properties shift across our gradient sites as well as throughout the growing season. We found that pH decreased going from low to high elevation ($p < 0.0001$, Figure 3) and increased throughout the growing season at Almont and Cinnamon ($p < 0.001$, 0.0001, Figures 5 and 7). Suprisingly, we found that soil conductivity did not change across our elevation sites or over time at Almont, but did decrease at Cinnamon over time ($p < 0.001$, Figure 6).
The final results of this study will compare the results of sequence analysis with the soil chemistry trends shown here, as well as trends shown in the ground cover, soil temperature, soil moisture, and slope.

Discussion

Not enough data have been collected at this time to merit a full discussion. However, the soil trends that have been found at this time useful for comparison to the sequencing data that will be collected in the fall. A pH gradient was shown to have an effect on bacterial dormancy rates, but not fungal dormancy rates, by Mueller (2016). Soil conductivity has not yet been compared to dormancy rates; however, since soil conductivity did not show a trend over an elevational gradient, it may not be useful for comparison to sequencing data. Overall, the current data show that dramatic trends exist over an elevational gradient and a time series, and that future analyses will be able to compare these existing trends to trends found in microbial genotypic data.

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Tables and figures

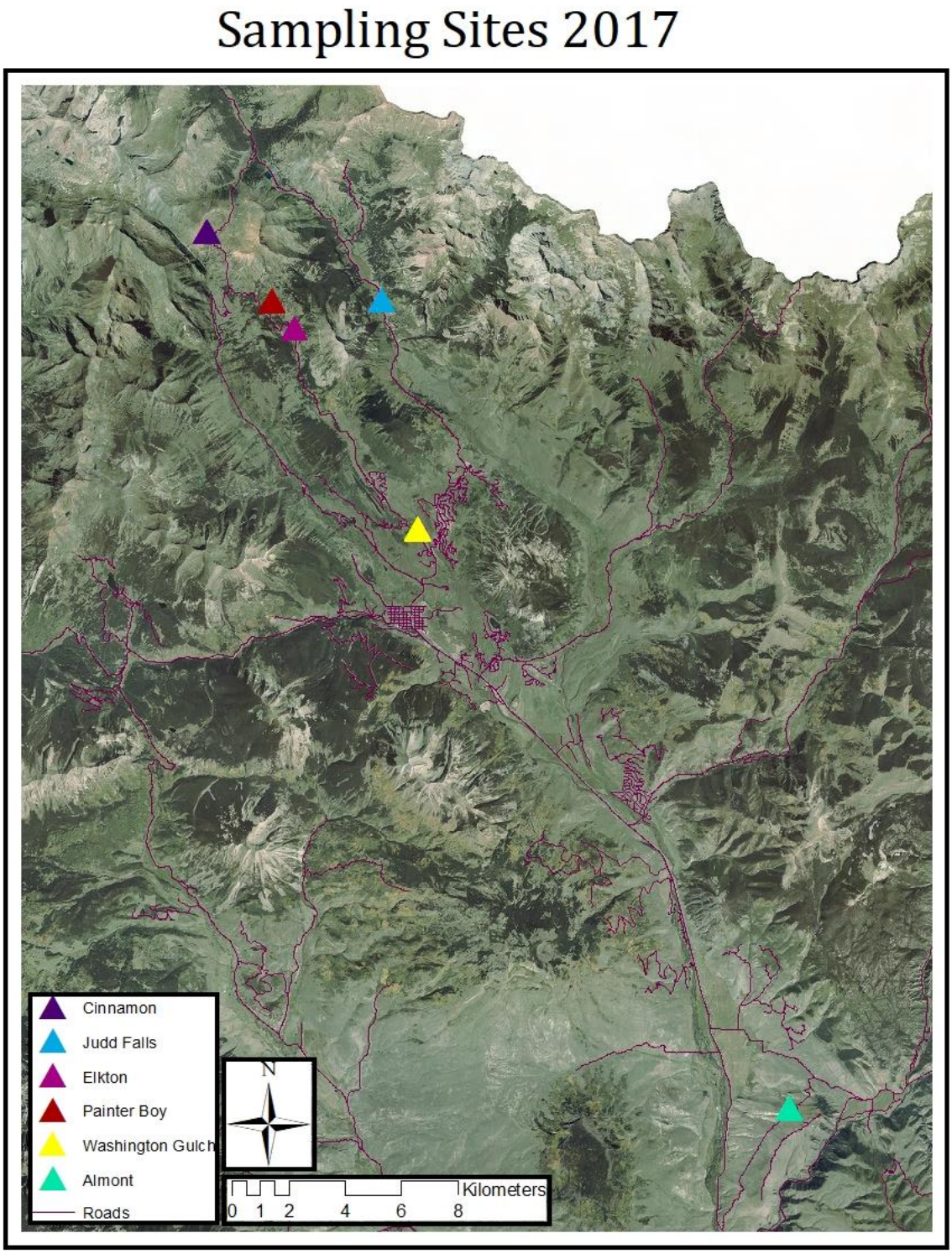

Figure 1. Map of sampling sites.

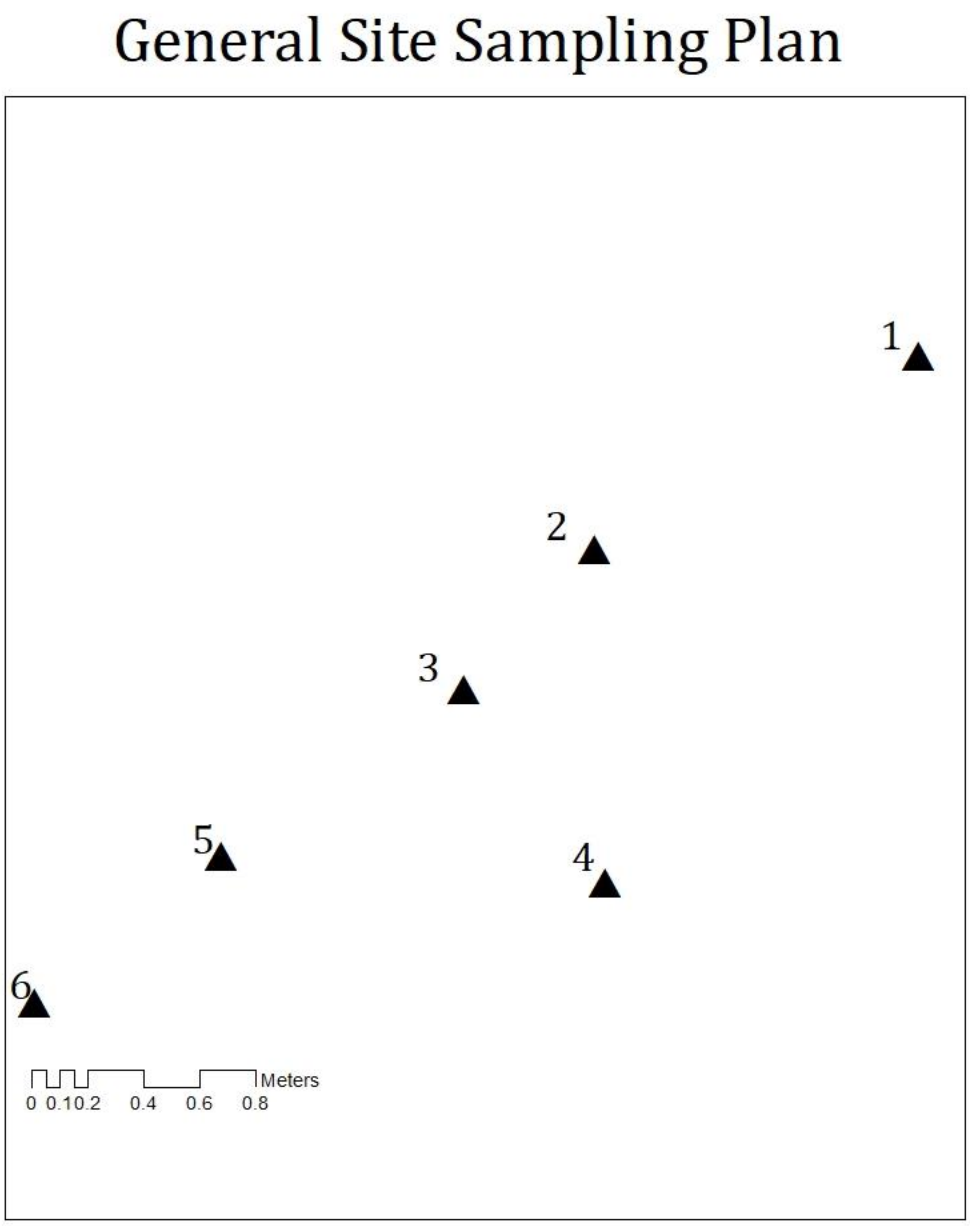

Figure 2. General site sampling plan.

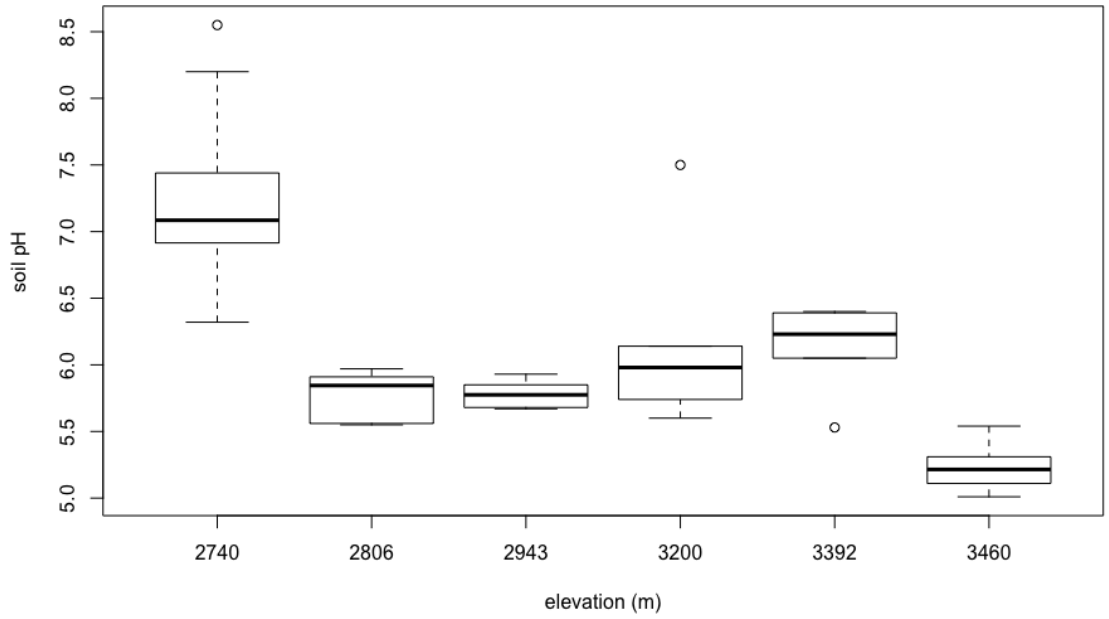

Figure 3. Soil pH over an elevational gradient.

Table 1. Anova Table of soil pH over an elevational gradient.

	Sum Sq	Df	F value	Pr(>F)
Date	34.51	1	3	6.01e-15
Residuals	24.64	70		

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

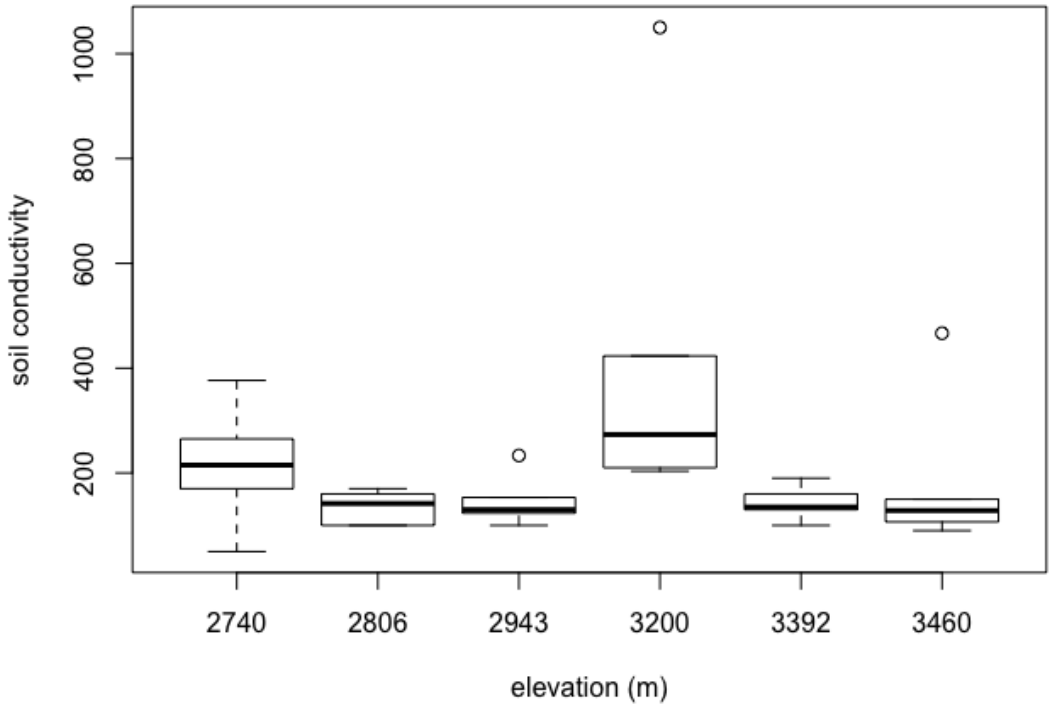

Figure 4. Soil conductivity over an elevational gradient.

Table 2. Anova Table of soil conductivity over an elevational gradient.

	Sum Sq	Df	F value	Pr(>F)
Date	70358	1	3.882	0.0528
Residuals	1250549	69		

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

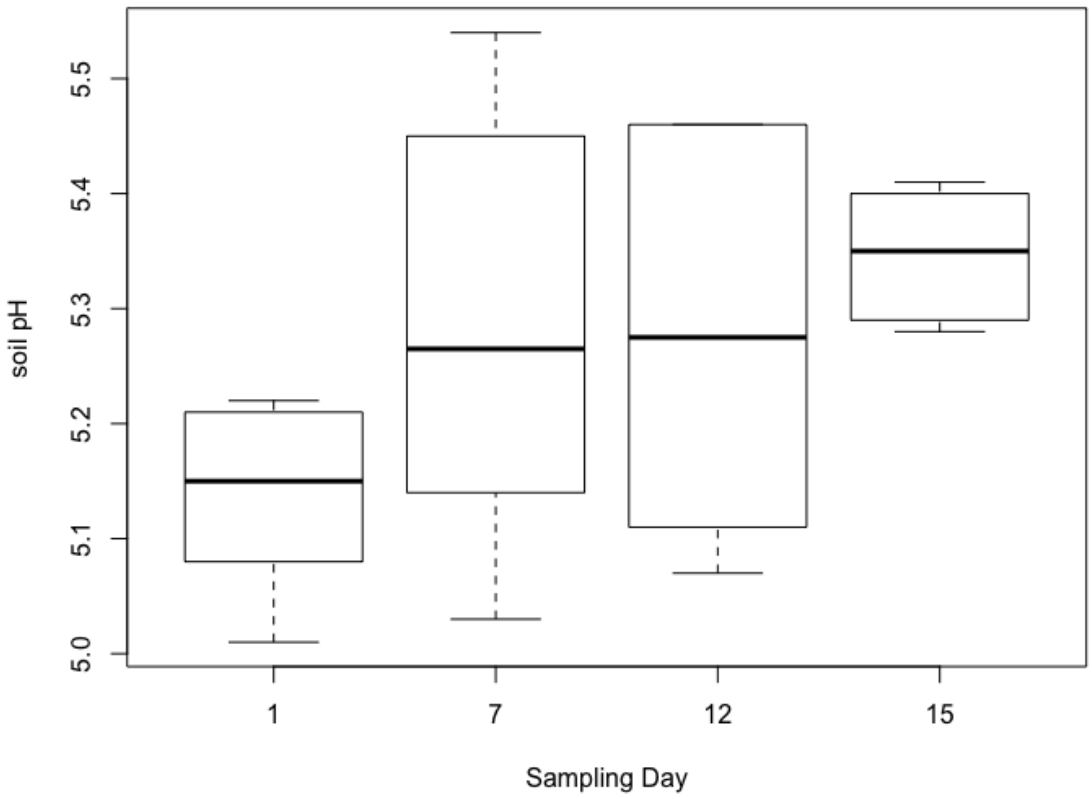

Figure 5. pH at Cinnamon over time.

Table 3. Anova Table of pH at Cinnamon over time.

	Sum Sq	Df	F value	Pr(>F)
Date	28.614	1	65.58	1.2e-11***
Residuals	30.54	69		

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

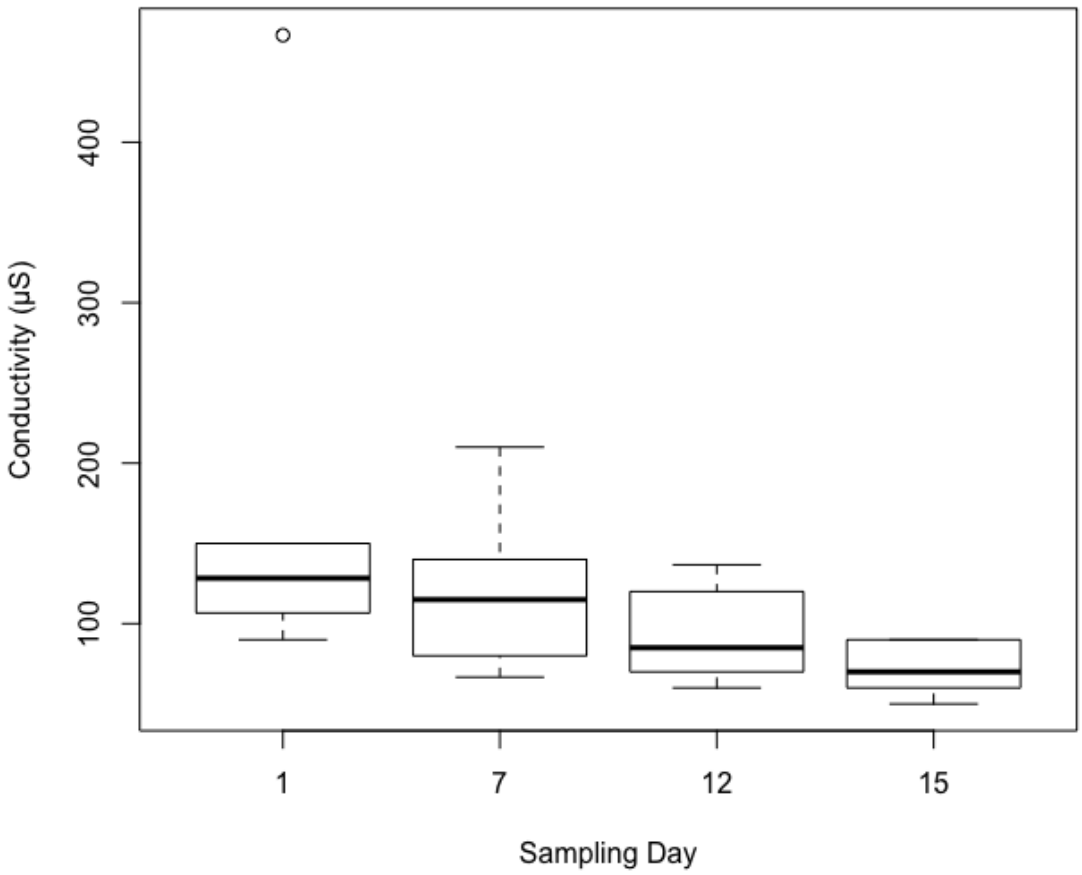

Figure 6. **Soil conductivity at Cinnamon over time.**

Table 4. Anova Table of soil conductivity at Cinnamon over time.

	Sum Sq	Df	F value	Pr(>F)
Date	155911	1	9.234	0.00335**
Residuals	1164997	69		

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

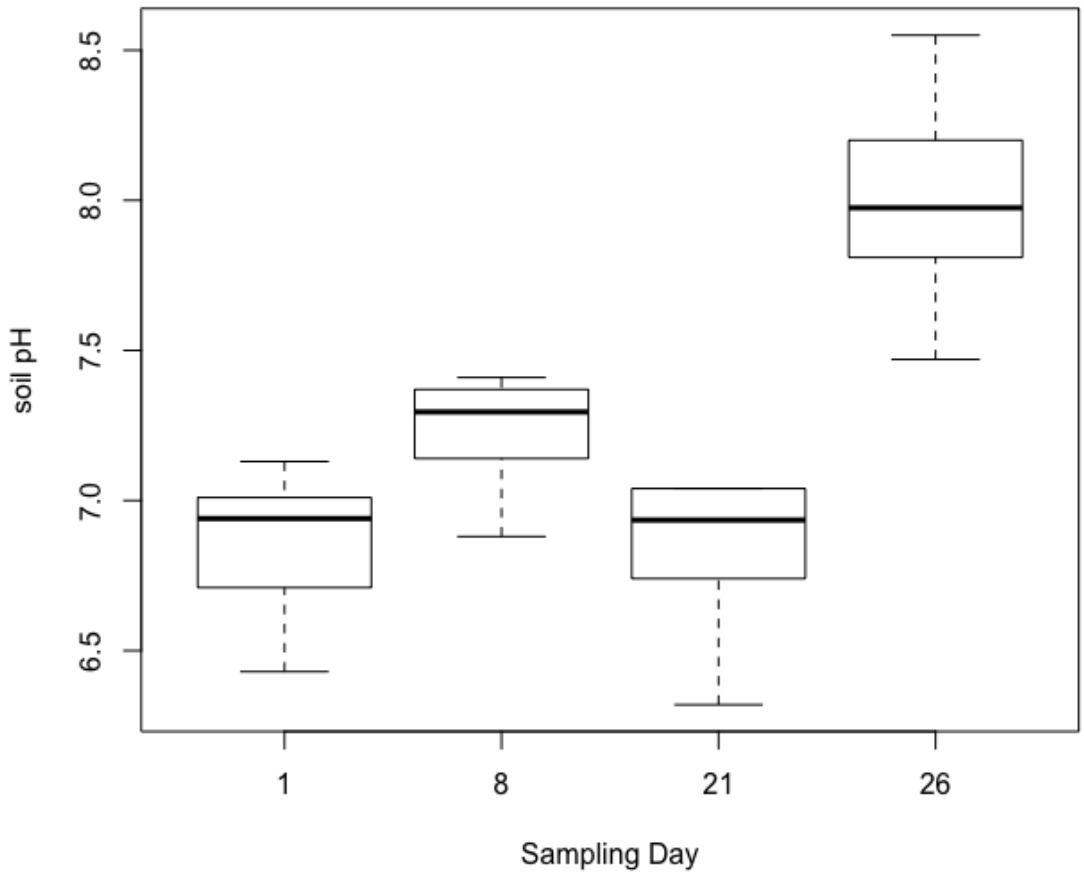

Figure 7. Soil pH at Almont over time.

Table 5. Anova Table of soil conductivity over an elevational gradient.

	Sum Sq	Df	F value	Pr(>F)
Date	70358	1	3.882	0.0528
Residuals	1250549	69		

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

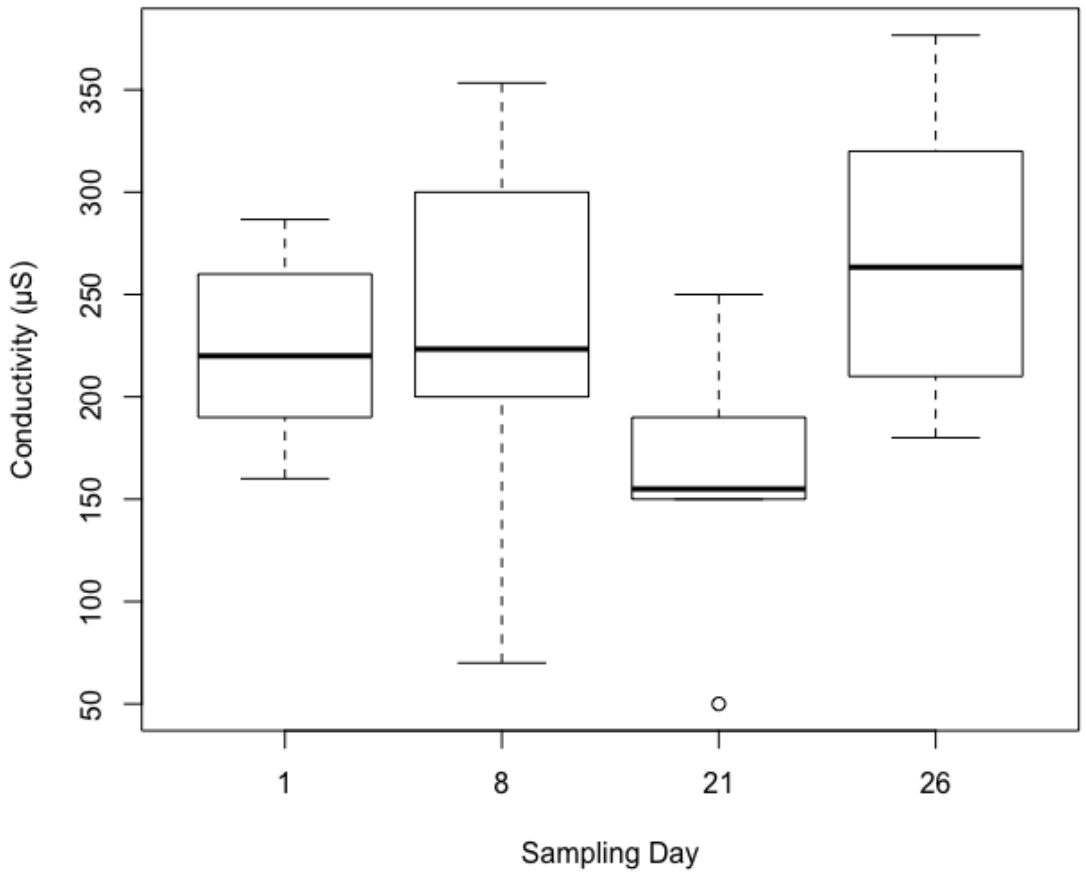

Figure 8. Soil conductivity at Almont over time.

Table 6. Anova Table of soil conductivity at Almont over time.

	Sum Sq	Df	F value	Pr(>F)
Date	52447	1	2.853	0.0957
Residuals	1268461	69		

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Figure list