A simple method for determining limiting nutrients for photosynthetic crusts


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A simple method for determining limiting nutrients for photosynthetic crusts


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Background: Photosynthetic crust communities are important to the functioning of many desert and early successional ecosystems. Little is known about the factors that limit the growth of these communities, especially during early stages of primary succession or following disturbance.

Aims: Our main goal was to develop a method to study nutrient limitations of crust growth in laboratory microcosms. We used the new method to test the hypothesis that phosphorus limits the growth of crusts in newly deglaciated soils of the high Andes.

Methods: We modified the point-intercept method used in plant ecology to quantify the spread of cyanobacteria, algae and mosses on the soil surface in response to additions of nitrogen and phosphorus.

Results: Fertilization with phosphorus significantly increased the growth rate and final percentage cover, and decreased the lag time for growth of cyanobacterial and algal communities in recently deglaciated soils. By contrast, nitrogen additions had no significant effect on the growth of microbial phototrophs, and all nutrient additions suppressed the growth of early successional mosses.

Conclusions: We propose that the method described here offers a valuable tool for assessing the nature of nutrient limitation of photosynthetic organisms in early successional and desert ecosystems. The information provided by using this approach can increase our understanding of the earliest stages of ecosystem development and may help inform strategies for the reclamation of disturbed arid ecosystems by identifying potential limiting nutrients.

Keywords: Glacial retreat; global warming; limiting nutrients; Peru; photosynthetic crust; primary succession

Introduction

Approximately 40% of the Earth’s terrestrial surface is covered by warm or cold deserts (Bowker et al. 2005), both of which often support diminutive photosynthetic communities, variously referred to as cryptogamic crusts and biological soil crusts, among many other names (Belnap 2003; Bowker et al. 2005; Davey and Clarke 1992; ZeIikova et al. 2012). These phototrophic communities are especially prevalent in areas that lack vascular plants, where they are able to exploit intermittent pulses of water to photosynthesise and grow (Belnap 2003; García-Pichel and Pringault 2001). Beyond water limitation, little is known about the soil nutrients that might further limit the growth of these communities (Belnap 2003; Bowker et al. 2006); however, there have been correlative studies indicating that micronutrients such as calcium (Ca), manganese (Mn), sulphur (S) and zinc (Zn) may influence the distribution and abundance of some crust-forming organisms (Bowker et al. 2005; Ullmann and Bödel 2001). In addition, some studies indicated that nitrogen (N) additions may adversely affect mosses in desert crusts (Stark et al. 2011) and in montane ecosystems (Pearce et al. 2003), but according to Bowker et al. (2005), no work has directly examined N or phosphorus (P) limitation of crust growth in desert ecosystems. There is also an obvious gap in the literature concerning experimental evidence for N or P limitation of photosynthetic microbes in soils in an early phase of development; however, based on previous studies of soil heterotrophs in glacial forelands (Göransson et al. 2011, Schmidt et al. 2011b; Yoshitake et al. 2007), it is possible (if not likely) that the lack of available N or P may limit the establishment and growth of biological soil crusts during primary succession.

Most of what is known about nutrient limitation during primary succession is derived from plant ecological studies (Walker and Syers 1976; Chapin et al. 1994; Matthews 1992; Vitousek 2004) that indicated that N most often limits plant productivity during early primary succession (Walker and Syers 1976; Vitousek 2004). Support for this hypothesis comes from the observation that all of the elements (except N) required for plant growth are usually found in geologic substrates on which primary succession occurs (Jenny 1980; Tilman 1988); however, while phosphorus (P) may be present in early successional soils or bedrock, it is often bound in unweathered minerals and is therefore unavailable to organisms (Schlesinger et al. 1998; Walker and Syers 1976). For example, recently deglaciated soils of the high Andes contain relatively high concentrations of P in primary mineral forms (≥600 μg g⁻¹) but very small stocks of biologically available P that can be accessed...
by heterotrophic microbes (<1 µg g⁻¹; Schmidt et al. 2011b). In addition, recent studies of terrestrial microbial communities in early and late successional ecosystems have indicated that P can limit microbial activity and growth (Cleveland et al. 2002; King et al. 2008), but these studies have been confined to the effects of P on heterotrophic processes (e.g. soil respiration). We do not know if phototrophic microbes have access to the same pools of P as heterotrophic microbes (Cleveland and Liptzin 2007; Reimers 1986) or if they are limited by P or N, or both (or neither) early in succession. This shortcoming limits our understanding of the earliest stages of ecosystem succession because cyanobacteria and other microbial phototrophs are among the dominant organisms that colonize recently deglaciated soils (Kaštovska et al. 2005; Nemergut et al. 2007; Schmidt et al. 2011a, Yoshioka et al. 2010).

Here we describe a simple method to determine the effects of added nutrients on photosynthetic microorganisms in early successional soils. Our approach was to microscopically, and non-destructively, monitor the spread of photosynthetic colonies on the soil surface in response to added nutrients in laboratory microcosms. We used theory and adapted methods from plant ecology (Bonham 1989; Jonasson 1988) and soil microbiology (Bowker et al. 2002; Schmidt et al. 2011b) to estimate the percentage cover, growth rates and lag times for development of photosynthetic microorganisms and mosses on the surface of recently deglaciated soils. We used this approach to determine if microbial phototrophs were limited by P or N in recently deglaciated soils in the high Andes of Peru. These early successional soils were just starting to form crust-like communities when collected (Schmidt et al. 2008a, see Materials and methods for details). Based on our previous work showing very low levels of microbe-available P (Schmidt et al. 2011b), and high potential for N fixation at this site (Schmidt et al. 2008a), we hypothesised that P was more limiting than N in this early successional ecosystem.

Materials and methods
Site and organism descriptions
The soils used in this study were collected in the rapidly deglaciating valley of the Sibinacocha Watershed in the Cordillera Vilcanota, Peru (13°46'24"S, 71°04'17"W; Schmidt et al. 2008a, 2008b; Seimon et al. 2007). These early successional soils are extremely oligotrophic and remain mostly devoid of plants (and macroscopic soil crusts) for many years after glacial retreat (Schmidt et al. 2008a). Biological soil crusts slowly form on these soils over a period of ca. 20 to 50 years (Nemergut et al. 2007; Schmidt et al. 2009), but soil darkening is seen in wetter areas within the first four years post-glacial retreat. The soils used in the present study were from sites that had been deglaciated in the previous four years and had not yet formed visible crusts but contained the propagules for crust formation, including a high diversity of cyanobacteria and algae, many of which have not been cultured or described (Nemergut et al. 2007; Schmidt et al. 2008a; Schmidt et al. 2011a). For example, 13 of the 29 cyanobacterial phylotypes in these soils fall into three deeply divergent clades (at the order or higher level) that contain only undescribed cyanobacteria from cold deserts of the Himalayas and Antarctica (supplemental Figure S2 in Schmidt et al. 2011a). The rest of the cyanobacteria in these soils fall into widely divergent groups including the Nostocales, Oscillatoriales and Pseudanabaenales (Schmidt et al. 2011a). The most abundant phylotype is a Nostoc sp. (GenBank: GQ300670) related to a free-living strain of N. sphaeroides (Lücking et al. 2009). Other abundant phylotypes included close relatives of a Himalayan strain (HQ189014) of Microcoleus vaginatus (Schmidt et al. 2011a), a lichen-forming Nostoc strain (EF174228) from Chile (Elvebakk et al. 2008), and a Phormidium-like isolate (DQ493873) from the high Arctic (Comte et al. 2007). The mosses of these high elevation soils have not been studied previously but they are upright (acrocarps) and resemble mosses in the Pottiaceae from high volcanoes and Antarctica (Schiavone and Suárez 2009); however, no sporophytes have been observed, nor has molecular work been reported, so positive identification of the mosses is not possible at this time. The crusts that eventually forms along the chronosequence are ‘smooth’ to ‘rugose’ to ‘rolling’ using the classification scheme of Belnap (2003).

Data collection and analysis
Soil samples (top 5 cm of soil) were collected in an area that had been uncovered by ice within the four years prior to collection and were within 100 m of the glacial terminus (Nemergut et al. 2007). Soil samples were frozen (−10°C) in the field (Schmidt et al. 2009) and then frozen to −20°C in Lima, Peru before being transported on ice to the University of Colorado, Boulder. Soils were stored at −20°C and thawed for 24 h prior to the beginning of the experiments and were then thoroughly homogenised and sieved (2.36 mm mesh size). Sieving removed only rocks as no established mosses or other macroscopic biological materials were visible in these early successional soils. The water holding capacity of the homogenised soil was determined as described elsewhere (Colores et al. 1996). Equal weight of soil (17.3 g dry weight) was added to all ‘microcosms’ (three replicates per treatment) in small sterile Petri plates (60 mm diameter × 15 mm high, Fisher Scientific 8-757-13A) resulting in a 3-4 mm deep layer of soil. N was added (in the initial watering of the soils) to the +N and +N+P treatments as NH₄NO₃ to obtain a final concentration of 75 µg N g⁻¹ soil. P was added (in the initial watering) to the +P and +N+P treatments as a mix of dibasic and monobasic phosphate to achieve a final concentration of 75 µg P g⁻¹ soil. The pH of the phosphate solution was adjusted to the pH of the soil (Nemergut et al. 2007) before addition. The concentrations of N and P were chosen based on the work of King et al. (2008). In order to ensure adequate water and prevent water logging, all soils were initially amended to 70% of water-holding capacity and were watered with sterile-deionised water every three days to 70% of water-holding capacity by weight (Scow.
et al. 1989). The arrangement of the plates was randomised after each watering in order to control for any variation in light and temperature.

Microcosms were initially incubated in an incubator (Sheldon Manufacturing Inc., Cornelius (OR)) under conditions mimicking the freeze-thaw cycles that these soils experience in the field (Schmidt et al. 2009), with temperatures dropping to 0 °C at night and rising to just above 25 °C during the day. After some growth of the native organisms (no inoculum was added) was evident in the microcosms (nine days), they were incubated at 22–28 °C on a laboratory bench for the remainder of the experiment. Supplemental lighting was provided by a reflective lamp with a 100 W broadspectrum (including UV) bulb (Zoo Med Laboratories, Inc.). Photoperiod was 15 h of light and 9 h of darkness. Data loggers (Hobo Pendant, Onset Computer Corp., Bourne (MA)) were kept with the microcosms at all times to record both temperature and light data.

Growth of photosynthetic colonies (green mats or filaments) on the soil surface was quantified non-destructively using a microscope (with illumination from above) and a point-intercept approach (Bonham 1989; Jonasson 1988). Once experiments were started, every microcosm was examined every three to four days by running multiple transects across each plate and determining the presence or absence of phototrophs in each field of view until at least 50 fields of view (45× magnification) were examined for each replicate at each sampling time. This method is adequate to estimate the percentage cover of colony-forming algae, moss and cyanobacteria, but does not allow for the quantification of unicellular photosynthetic bacteria (which were not examined in this study). Preliminary tests showed that total percentage cover of phototrophs was proportional to the percentage of microscopic fields containing phototrophs as long as at least 50 microscopic views were recorded for each microcosm at each sampling time. This level of sampling is equivalent to randomly examining 35% of the total surface area of each microcosm at each sampling time. A standard curve was generated from separate microcosms ($n = 12$) to those used in the study, and used to convert the percentage of microscopic fields to percentage cover: % cover = 0.937 (positive microscope fields) ($R^2 = 0.96$). This standard curve is only valid up to the point at which percentage cover is equal to 45% of the total surface area, after which the percentage of microscopic fields cannot be used to accurately estimate percent cover because the relationship between the two variables reached an asymptote. Higher values of percentage cover can be estimated if a higher magnification is used because the size of the microscope field more closely approximates a point in space at each higher level of magnification. Higher levels of magnification were not necessary in the present study because the density of phototrophs was low. Percentage cover for soils with higher density of phototrophs can be determined by using a more tedious version of the point-intercept method in which crosshairs on the ocular lens is used as a true point. Fifty to 100 points need to be examined along random transects of each microcosm to obtain reliable estimates of cover, but this procedure was not necessary in the present study.

A two-way ANOVA with interaction and corrected for repeated measures (Devore 2004) was performed on the growth data (see Figure 1) using R (http://www.r-project.org/index.html). Percentage cover was modelled by time, and treatment, with an error term for the treatment replicates. Interaction between time and treatment was included in the model. The repeated measures approach was needed because measurements of a single microcosm over time are not independent of each other. Growth rates were estimated by linear regression of growth versus time for the linear portion of each data set (after 600 hours the +P and +P+N treatments levelled off at the maximum percentage cover values shown in Figure 4). A two-way ANOVA with interaction and corrected for repeated measures showed high levels of overall significance $P < 0.0001$ and significant differences between all treatments on most dates (Tukey's Honestly Significant Differences test).

Results

Overall our results showed that P was more limiting than N to soil phototrophs under the experimental conditions used in the present study. The addition of P allowed the growth of phototrophs to begin sooner, grow faster and reach a higher percentage cover than the treatment just receiving N or the control (see Figure 1). The stimulatory effect of P resulted in significantly higher growth rates (see Figure 2; $P < 0.05$) and significantly reduced lag times (see Figure 3; $P < 0.005$) in the +P and +P+N treatments compared to the control and +N treatment. For example, the growth rate was increased by 71% and the lag time was reduced...
by 97% in the +P treatment compared to the control (see Figures 2 and 3). Likewise, the maximum percentage cover reached during the experiment was significantly higher in microcosms receiving P (see Figure 4; \( P < 0.05 \)), being 31% higher in the +P treatment compared to the control.

Our approach also allowed us to monitor the effects of nutrients on larger organisms in the microcosms. Unlike microbial phototrophs, moss growth was inhibited by all nutrient additions compared to the water-only control (see Figure 5).

**Discussion**

The main goal of this research was to develop a simple method to determine if and what nutrients limit the growth of photosynthetic microbes during primary succession in glacier foreland soils. Although we developed the method to examine early successional soils, it could also be applied to any soils that have populations of microbial phototrophs, such as desert crust (e.g. Bowker et al. 2005; Zelikova et al. 2012), or geologically old, unvegetated soils of alpine, Arctic and Antarctic environments (e.g. Freeman et al. 2009; Fell et al. 2006). One advantage of this approach is that it requires minimal equipment, just a dissecting...
Another intriguing finding of the present study is that unlike microbial phototrophs, mosses were inhibited by all of the nutrient addition treatments (see Figure 5). This finding could indicate that the unidentified moss species present in these soils are extremely well adapted to low nutrient conditions and are therefore inhibited by high nutrient availabilities, as has been suggested for lichens from oligotrophic systems (Welch et al. 2006) and for mosses in various ecosystems (Li and Glime 1990; Stark et al. 2011). Mosses in tundra ecosystems have also been reported to respond negatively to additions of both N and P (Chapin and Shaver 1985; Nemergut et al. 2008), but mechanisms for this inhibition have not been determined. The fact that mosses are inhibited by nutrient additions makes it unclear if the present method is useful for determining what, if any, nutrients are limiting to their growth. Future work with mosses should include using lower concentrations of nutrient or different chemical species to ensure that inhibition by particular ions is not masking any potential growth stimulation that could occur from nutrient additions.

Finally, this work highlights the importance of carrying out basic, manipulative experiments in order to gain a preliminary understanding of the roles of microbial communities in a broader ecological context. Our finding of P limitation in microcosms of early successional soils is just a first step towards understanding what limits microbial growth in the field and some of our future effort will be directed at studying this phenomenon in the field at the remote Andean sites where these soils originated.

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References


