Microbial Ecology

Nitrogen Fixation and Leaching of Biological Soil Crust Communities in Mesic Temperate Soils

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Abstract

Biological soil crust is composed of lichens, cyanobacteria, green algae, mosses, and fungi. Although crusts are a dominant source of nitrogen (N) in arid ecosystems, this study is among the first to demonstrate their contribution to N availability in xeric temperate habitats. The study site is located in Lucas County of Northwest Ohio. Using an acetylene reduction technique, we demonstrated potential N fixation for these crusts covering sandy, acidic, low N soil. Similar fixation rates were observed for crust whether dominated by moss, lichen, or bare soil. N inputs from biological crusts in northwestern Ohio are comparable to those in arid regions, but contribute substantially less N than by atmospheric deposition. Nitrate and ammonium leaching from the crust layer were quantified using ion exchange resin bags inserted within intact soil cores at 4 cm depth. Leaching of ammonium was greater and nitrate less in lichen than moss crusts or bare soil, and was less than that deposited from atmospheric sources. Therefore, biological crusts in these mesic, temperate soils may be immobilizing excess ammonium and nitrate that would otherwise be leached through the sandy soil. Moreover, automated monitoring of microclimate in the surface 7 cm of soil suggests that moisture and temperature fluctuations in soil are moderated under crust compared to bare soil without crust. We conclude that biological crusts in northwestern Ohio contribute potential N fixation, reduce N leaching, and moderate soil microclimate.

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Introduction

Comprised primarily of cyanobacteria, algae, lichens, and moss at or near the soil surface, biological soil crusts play essential roles in soil stability and nutrient cycling. Crust communities contribute organic matter, nutrients, and physical stability to soils in extreme microhabitats [1, 6, 24]. Depending on the type of biological crust, combinations of fungal hyphae, cyanobacterial filaments, and rhizoids of lichens and mosses may create a mesh throughout the surface layer of the soil, physically intertwining soil particles and aggregates, and reducing wind and water erosion [2, 5, 7, 20, 31]. In addition, biological crusts have a rough surface microtopography [6] that diminishes the impact of surface runoff and wind. Where larger crust organisms such as mosses, liverworts, and lichens cover the soil, raindrops cannot directly impact the surface and detach soil particles. In turn, this matrix increases soil moisture retention [14], which may [6, 24] or may not [20, 22] enhance seed germination of herbaceous plants.

Biological soil crusts with cyanobacteria and cyanolichens are capable of fixing significant quantities of atmospheric dinitrogen (N_2) into ammonium (NH_4^+). Estimates of N input by biological soil crusts vary considerably. For example, West and Skujiņš [40] estimate that cyanobacteria in crusts contribute 25–100 kg N ha^{-1} annually in the northeastern corner of the Great Basin desert (northwestern Utah). In contrast, cyanobacteria contribute only 7–18 kg N ha^{-1} $year^{-1}$ in the Sonoran Desert (southern Arizona, 34) and 0.02–3.6 kg ha^{-1} $year^{-1}$ in southern Utah [23]. Cyanobacteria and lichens release 5–70% of total N fixed, depending on soil moisture, temperature, light intensity, and season [3]. This represents a significant portion of N input in desert ecosystems [37, 39, 40]. In contrast to desert environments, atmospheric deposition can be a major contrib-

Table 1. Levels of nitrogen (kg ha^{-1}) accumulated per year by precipitation and per season[a] for soil concentrations and leaching concentrations at Oak Openings, Lucas County, OH

	Nitrate			*Ammonium*		
	1999	*2000*	*2001*	*1999*	*2000*	*2001*
Wet deposition	13.20	17.92	–[b]	2.90	6.80	–
Soil	0.24 to 0.92	–	4.06×10^{-5} to 1.5×10^{-2}	0 to 0.23	–	5.2×10^{-4} to 1.8×10^{-2}
Leaching	–	1.1×10^{-2} to 5.7×10^{-1}	2.6×10^{-5} to 4.3×10^{-2}	–	5.9×10^{-3} to 1.04	3.6×10^{-3} to 2.0×10^{-2}

Wet deposition is expressed as a mean, and soil and leaching are expressed as ranges (minimum to maximum values). Estimates of wet deposition were determined by performing punctual kriging analysis on data available from the National Atmospheric Deposition program (http://nadp.sws.uiuc.edu/), collected at 36 stations within a 450-km radius of the study site [38].
[a] Each spring, summer, autumn, and winter were computed as 3-month periods.
[b] Not measured.

utor to soil N in mesic climates [15]. Atmospheric deposition must pass through crusts before reaching the underlying soil. Therefore, crusts may regulate capture and release of atmospheric N [20].

Crust communities have been extensively studied in arid and semiarid lands throughout the world, where they may constitute more than 70% of the living cover [8]. Soil-crust communities are less extensive, yet occur in xeric patches of temperate regions, such as steppe formations in central Europe, sand dunes, and pine barrens of the United States [3, 20, 21, 22, 36], where vascular plants are absent or scarce. Although several reviews on biological soil crusts are published [8, 13, 16], relationships of these crust communities to ecological functions of arid patches where prevailing climate is mesic and temperate is little known [20, 22]. Identifying ecological roles of these crusts may influence land management practices. Crusts in northwestern Ohio are primarily composed of mosses and lichens; none of the lichens contain cyanobacteria as their photobionts [31]. These contrast with the composition of crusts in both Florida shrublands where algae and cyanobacteria dominate rather than mosses or lichens [20, 21] and arid land crusts are generally dominated by lichens with cyanobacteria phycobionts [33] or free-living cyanobacteria. The primary goal of this study was to test the hypothesis that areas with well-established biological soil crusts fix more N and, therefore, leach more N than areas with bare soil or less-established crusts. Alternatively, crusts might compete with plants for available N, reducing leaching or loss of N [20]. A secondary goal was to determine whether crust establishment affected N fixation or leaching indirectly by altering microclimate.

Methods

Study Site. A field experiment was conducted in the Badger Barren in Oak Openings Preserve Metropark (Swanton Township, Lucas County) in northwestern Ohio [31] in 2001. Hereafter, the site will be referred to as Oak Openings. The site is classified as a dry sand savanna (sand barren) with acidic sandy soils, low available water holding capacity, and low inorganic nutrient availability [41]. Annual precipitation at our site during the study period was 73–97 cm. Within that wet atmospheric precipitation, 13.2–17.9 kg of NO_3^- and 2.9–6.8 kg of NH_4^+ per hectare were deposited at our study site annually (Table 1). Species composition of biological crust components (cyanobacteria, bryophytes, lichens, chlorophyta, and bacteria), soil fauna (nematodes, collembolans, mites), and vascular plants has been published [31].

Experimental Design. On May 16, 2001, 99 pairs of intact soil cores (4 cm diameter, 7.5 cm deep) were obtained from the study site (0.5 ha) in polyethylene soil tubes. Each pair of cores represented an experimental unit, a member of each pair was used for monitoring leaching of NO_3^- independently from NH_4^+ to avoid interference associated with mixed-bed exchange resins [9]. Locations of experimental units were chosen, proportional to land area, to represent three categories of crust establishment: late (moss-dominated), intermediate (lichen-dominated), and early (bare soil) successional (Fig. 1, Table 2).

Classes of crust establishment were based on video image analysis of each experimental unit. Immediately upon core removal, a photograph of each intact soil core surface (12.6 cm^2) was taken using an Olympus OM-1 camera with a macro lens, tripod, and flash assembly. Each slide was scanned using an Acer Scanwit 2720S film scanner (San Jose, CA) and saved as a digital image. Images were analyzed using Zeiss KS300 video-imaging software (Axiovision 2.0, Carl Zeiss Vision GmbH, Hallbergmoos, Germany). A software macro for the KS300 software was used to improve image clarity by removing shadows. Each type of ground cover was outlined manually for classification and quantification of crust cover (cm^2 of total surface area and converted to proportion cover). Types of ground cover quantified

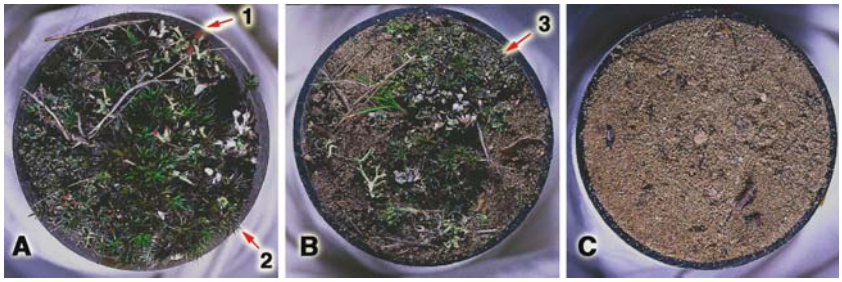

Figure 1. (A) Moss (late successional) crust, (B) lichen (intermediate successional) crust, and (C) bare soil (early successional crust). Total extent cover and composition was determined by video-imaging analysis. *Arrows* indicate (1) lichen, (2) moss, and (3) cyanobacteria/algae.

were moss, lichens, cyanobacteria and algae, and bare soil. Each cover type was distinguished based on color and texture defined in preliminary experiments. After photographing the surface, a snug-fitting cap was placed on the top end of the tube to protect the integrity of the crust and a resin bag was inserted into each core center. The center position was chosen to ensure that ions on the resins were from soil directly above the bags and to avoid preferential flow around or beneath the core, determined from a preliminary experiment [38]. Each core received a bag containing 2.65 g dry weight of loose ion exchange resin beds. Amberlite IRN-78 and Amberlite IRN-77 (Rohm and Haas, Inc., Philadelphia, PA, USA) resins were chosen for their relative affinities to NO_3^- and NH_4^+, respectively. Resin bags (4×4 cm^2) were constructed with No-See-Um® polyester netting (The Rain Shed, Inc., Corvallis, OR, USA) and exposed edges closed by heat sealing. Resins were rinsed with their respective charging solutions prior to installation and rinsed in ultrapure water, and always handled with forceps to avoid contamination. To install a resin bag into each core, 5 cm of bottom soil was removed with a spatula, a bag was carefully placed flush with the remaining soil using forceps, and then removed soil was replaced by layer and pressed to fill gaps between resin surface and soil close to original bulk density (1.51 mg m^{-3}). Each core was replaced to its origin, flush with the soil surface (*sensu lato*, [12]) and was incubated in field conditions at Oak Openings.

Sampling. Every 2 weeks after cylinder installation, five pairs of cores were harvested. At each harvest, potential N fixation and N leaching were measured respectively using an acetylene reduction (AR) method and separate extraction of NO_3^- and NH_4^+ from resin beads. AR detects activity of nitrogenase, capable of catalyzing both the reactions of acetylene to ethylene and N_2 to NH_4^+ [19]. To measure potential N fixation, a subsample (2 cm depth) was carefully removed from each core using a small beveled-edge plastic incubation tube (50 cm^3). Each sample was enclosed in the incubation tube (10.3 cm tall, 2.5 cm inside diameter) the atmosphere within the tube adjusted to approximately 10% acetylene, and incubated under ideal conditions for 4 h [2]. After incubation, 250 µl of gas from each tube was injected into a Shimadzu 17A gas chromatograph. The GC was fitted with a packed column OPN RES-SEL-C that had the following dimensions: 3.7 m (length), 2 mm (ID), 3.2 mm (OD), and 80/100 mesh. GC conditions for injection and detection were set for an isothermal temperature of 200 °C with helium as a carrier gas at 30 mL min^{-1}, and a run time of 4 min. Three controls were included: a positive control for 10% acetylene, a positive control for a mixture of ethylene and acetylene, and a blank. Standards were analyzed after every 10 injections for quality assurance. Estimates for the blank were subtracted from treatment values to correct for any background explained by the environment or instrument. Potential N fixation rate was expressed as mmol ethylene produced per square centimeter per hour.

To measure N leaching, resin bags were removed from centers of each core, and the soil contiguous to the resin bag was carefully isolated for independent analysis of N content. The period from harvest in the field to extraction in the laboratory never exceeded 2 h, to avoid dehydration of bags, contamination, or disturbance. To optimize extraction of loaded ions from information exchange requirements (IER) matrix, we modified the manufacturer's procedure as follows, based on a series of preliminary experiments. Each resin type and experimental unit was treated individually, agitated on a horizontal shaker [28]. Specifically, IRN-78 resins were rinsed or extracted by agitation in a starting solution of 25 mL

Table 2. Mean (±standard error) percentage of core surface (12.6 cm^2) of each crust cover class covered by moss, lichen, algae, or bare soil

Succession cover class	*Moss*	*Lichen*	*Algae*	*Bare*
Late (n = 64)	33.2 (±3.2)	31.2 (±2.7)	7.8 (±1.6)	27.9 (±2.1)
Intermediate (n = 85)	9.5 (±1.1)	11.3 (±1.1)	11.2 (±1.0)	68.0 (±1.3)
Early (n = 47)	0.2 (±0.1)	0.4 (±0.1)	2.3 (±0.4)	97.2 (±0.4)

1 N potassium bisulfate ($KHSO_4$). To counteract an immediate increase in its pH, acid aliquots (1 N H_2SO_4) were added gradually to bring the solution to an equilibrium pH of 1.5, the dissociation constant for $KHSO_4$. IRN-77 resins were rinsed or extracted by agitation for 30 min in 30 mL of 1 N potassium sulfate (K_2SO_4) and no pH adjustment was necessary. Recovery efficiency for 0.133–1.33 μg g^{-1} of NO_3^- and NH_4^+ ions from resins was 33% and 70%, respectively, under optimal conditions (Table 3). The filtrates from resins and soils (solution with expected NO_3^- or NH_4^+ ions) were kept in sealed containers and stored in a freezer for no longer than 3 weeks prior to colorimetric analysis. Availability of NO_3^- and NH_4^+ were quantified according to the procedure described by Cataldo *et al.* [11] and an indophenol blue method with EDTA [26], respectively. The lowest threshold of detection without interfering ions was 1.25 μg g^{-1} of N. Soil N content was standardized by dry weight.

Two supplemental measures of primary production and N fixation were performed, surface reflectance of photosynthetic pigments and abundance of cyanobacteria. First, crusts with contrasting composition were also characterized physically by reflectance of surfaces. Soil albedo was measured at 5 P.M. on July 26, 2001, with a portable spectroradiometer (GER-3700, Millbrook, NY, USA) with spectra ranging from 400 to 2500 nm (near infrared). A white ceramic plate was used as a reference standard for 100% reflectance during each measurement. Second, total number of cyanobacteria was quantified for three samples of moss crust collected on each September 26, October 3 and 24, 2001. Three subsamples per sample of moss-covered crust were analyzed, one at each of three depths, i.e., 0–3, 3.1–6.0, and 6.1–9.0 mm. Enumerations were made for four microscope fields per subsample (n = 108). Populations of cyanobacteria were expressed as cells per gram of dry soil using a hemacytometer and epifluorescence microscopy. Metabolically active cells with chlorophyll fluoresce as red or pink when viewed through a blue filter under ultraviolet light. With differential interference contrast, we could determine if fluorescing cells were algae or cyanobacteria, based on whether or not pigment was contained within chloroplasts [30].

Table 3. Mean (±standard error) recovery efficiency (n = 3) at varying concentrations of NO_3^- and NH_4^+ by anion exchange resins (extracted in 1 N $KHSO_4$ with pH 1.4) and cation exchange resins (extracted in 1 N K_2SO_4), respectively

Concentration (μg g^{-1})	NO_3^-	NH_4^+
0.133	13.3 (±1.9)	42.2 (±2.6)
0.22	17.3 (±2.1)	45.8 (±4.7)
0.33	21.2 (±2.9)	43.9 (±7.2)
0.66	22.8 (±2.7)	51.5 (±3.9)
1.33	33.9 (±1.8)	71.3 (±1.5)

Microclimate. Local climate was monitored in well-established crust and bare soil habitats. One Campbell Scientific (Logan, UT, USA) 10X data logger was placed in each habitat type. Soil moisture and soil temperature were measured at 7 cm depth using Watermark® sensors and thermistors, respectively. Hourly values for minimum, maximum, and average levels of both variables were recorded.

Statistical Analysis. Univariate fixed model ANOVAs were performed to determine the effect of crust establishment on potential N fixation rate, leaching of NH_4^+ or NO_3^-, and number of cyanobacterial cells at three depths in moss-covered crust. Statistics were performed using the MIXED procedure in SAS software Version 8 [35].

Results

Rates of N fixation by biological soil crusts in northwestern Ohio were similar for classes of establishment ($F = 0.17$, $p = 0.8454$) and occurred throughout the spring, summer, and fall (Fig. 2A). Abundance of cyanobacteria declined with depth in moss-covered crust (Fig. 3). In contrast, crust type affected N leaching (Fig. 4). Greater losses of NH_4^+ occurred under lichen crust than moss crust or bare soil ($F = 6.9$, $p = 0.0100$). The opposite pattern was observed for loss of NO_3^- ($F = 3.60$, $p = 0.0608$).

The presence of abundant crust cover buffered fluctuations in soil temperature and moisture more efficiently than bare soil. Soils covered by crust remained moister and cooler, experiencing oscillations with less amplitude than bare soils (Figs. 2B and C). Bare soils drained more quickly than soils with crust, attaining more negative water potential values than soils with crust. Nitrogenase activity was unaffected by fluctuations in temperature and moisture in the growing season.

Measures of soil albedo and photosynthetically active radiation differed among moss-covered crusts, lichen-covered crusts, and bare soil ($p = 0.007$). Moss-covered crust had the least reflectance (Fig. 5) because of the abundance of pigments causing the crust to appear darker. As moss coverage decreased, reflectance increased.

Discussion

Biologically available nitrogen (N) originates from atmospheric deposition, N turnover within the soil vegetation system, and biological sources (e.g., N fixation). We demonstrate that biological soil crusts in xeric patches of mesic climates are capable of fixing N

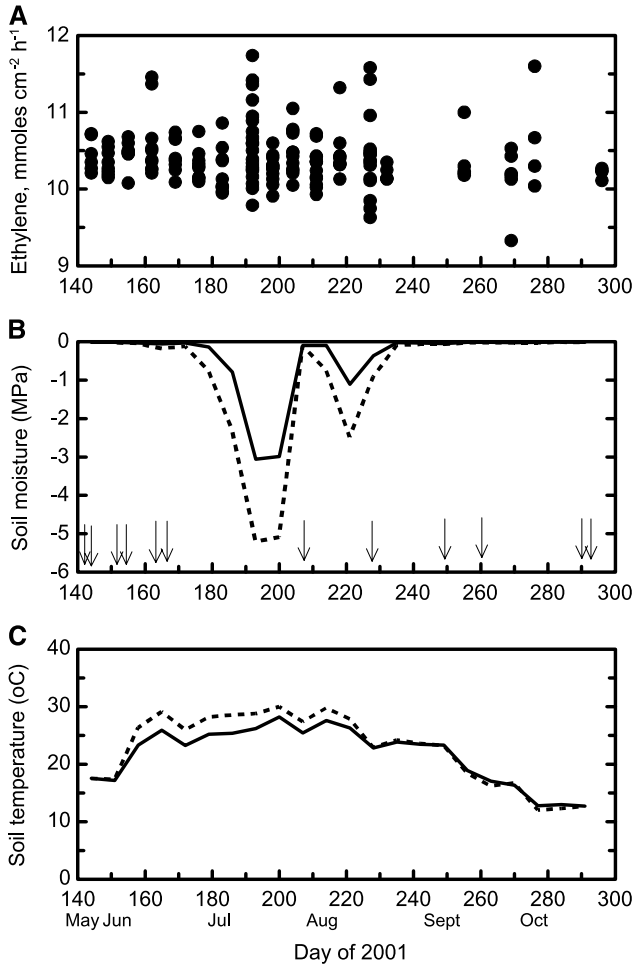

Figure 2. Mean (A) nitrogenase activity of biological soil crusts, (B) soil moisture, and (C) temperature (°C). Nitrogenase activity is measured in mmol ethylene resulting from acetylene reduction; samples were wet with 3 mL distilled water and incubated 4 h in a growth chamber with a constant relative humidity of 65%, temperature of 26 C°, and cool white fluorescent light (120 μmol s^{-1} m^{-2}). Symbols represent individual experimental units. Temperature and moisture were measured at 7 cm depth in soil covered by moss crust (*solid line*) and bare soil (*dashed line*), from May 24 to October 23, 2001. Soil moisture is expressed as water potential with MPa = 0 for saturated soils and MPa = −5 for extremely dry soil. Rainfall events are illustrated with a downward facing arrow in panel (B).

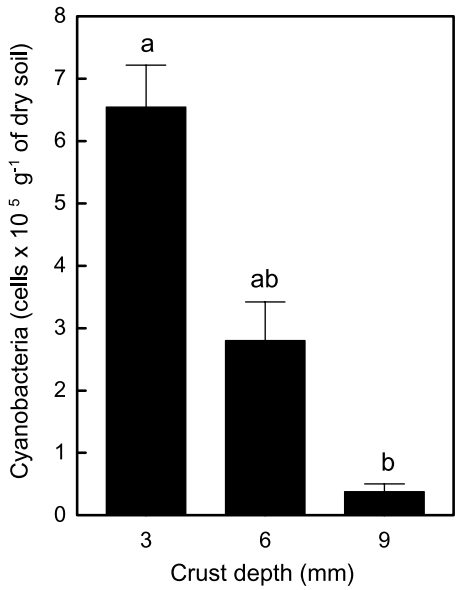

Figure 3. Abundance of cyanobacteria at three depths (0–3.0, 3.1–6.0, 6.1–9.0 mm) from the surface of moss-covered crust. Each bar represents a mean of four microscope fields for each of nine cores (n = 36). Standard errors of mean values are illustrated. Contrasting letters indicate statistical significance ($p \leq 0.0002$).

and potentially make it available for vascular plants, moss, and microorganisms. Converting our estimates of potential N fixation according to Boddey [10] and Hardy *et al.* [19] suggest that biological crusts of northwestern Ohio contributed a median of 1.3 kg ha^{-2} $year^{-1}$ of potential fixed N. These values are likely conservative estimates (see [29]) and exceed available nitrate and ammonium in soil or by leaching (Table 1). Therefore, crusts in an arid area of a mesic climate are contributing similar amounts of N through fixation as crusts in southern Utah than the Great Basin or Sonoran deserts [4, 23, 34, 40]. Quantitative comparisons should be made with caution, however, because multiple studies have shown the conversion ratio of ethylene to N is highly variable (0.1–6 for terrestrial species), depending on species and environmental conditions [17]. We applied a conversion rate of 3.2 reported for cyanobacteria [10], but conversion rates reported in the literature for the C_2H_4/N_2 ratio varies from 2 to 56 [3]. Environmental

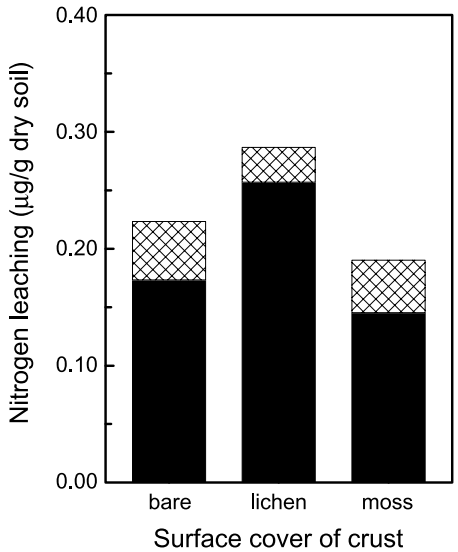

Figure 4. Mean leaching of NO_3^- (*hatched*) and NH_4^+ (*solid*) from soils covered by moss, lichen, or bare soil.

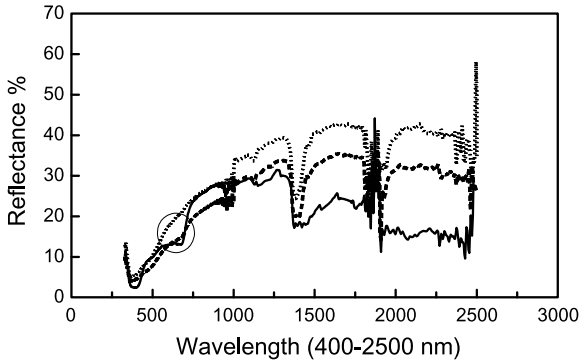

Figure 5. Soil albedo for three categories of soil cover: moss (*solid*) or lichen (*dashed*) crust covers, and bare soil (*dotted*). *Circled area* is called "red edge", which is a transition from low to higher reflectance at 670–780 nm.

conditions may lead to large variations in conversion rates. ^{15}N methods are necessary to provide absolute rates of N fixation.

Contrary to our hypothesis, potential N fixation was similar across all types of crust establishment at the study site. Perhaps this result occurred because cyanolichens did not occur at our site [31], and moss itself does not fix N. Of 12 lichen species at the study site, eight were *Cladonia* species and two were *Cladina* [31]. Although not quantified directly, we deduce that free-living cyanobacteria were ubiquitous. For example, we observed epiphytic cyanobacteria on moss-covered crusts, similar to Peters *et al.* [32]. Based on our methods, we can not distinguish whether observed N fixation in our study was performed by heterocystous cyanobacteria or other ammonia-oxidizing bacteria such as *Nitrosomonas* [25]. Johnson *et al.* [25] note that well-established crusts in desert soils contain heterocystous cyanobacteria (e.g., *Nostoc* and *Scytonema*) and less-established crusts do not (i.e., contain *Microcoleus*). Although species of epiphytic cyanobacteria were not identified, bare soil at Oak Openings contained some algal and cyanobacteria species that also occur in deserts of the southwestern U.S., notably *Microcoleus vaginatus*, *Desmococcus olivaceus*, and *Stichococcus bacillaris* [31]. Although the pH in our soils is much lower than those observed in the southwestern U.S. [8], mosses both secrete alkaline substances and use the high cation exchange capacity of their leaves to buffer pH of leaf surfaces [3], creating favorable conditions for epiphytic cyanobacteria.

A major difference between northwestern Ohio and the U.S. deserts is the relative contribution of N by atmospheric deposition. N deposition in western deserts is negligible making N fixation by biological soil crusts a primary source of N for plants [15]. At our study site, atmospheric deposition contributes more NO_3^- and NH_4^+ than N fixation by 1 order of magnitude. At our site, atmospheric wet deposition of NH_4^+ was similar to concentrations available in soil or by leaching (Table 1) and greater than those reported for Florida [20]. In contrast, atmospheric wet deposition of nitrate was similar to those observed in Florida [20] and exceeded concentrations available in soil or by leaching by 1 order of magnitude. This suggests crust-covered soils regulate losses of nitrate in these sandy soils.

Our original hypothesis on the effect of crust on leaching was supported only partially. Leaching of NH_4^+ was greater and leaching of NO_3^- was less in lichen crusts than moss crusts or bare soil. Bare soil in our study was dominated by algae and cyanobacteria and, perhaps, functioning similar to crusts in Florida noted to immobilize 20–40% of N added as a $^{15}NH_4{}^{15}NO_3$ tracer [20, 21]. A variety of processes can affect the concentration of NH_4^+ in the soil solution, including uptake by plants, immobilization by microbes, and fixation in clay minerals. Leaching of NH_4^+ could have been minimized by moss crust uptake or undergone nitrification, in which the oxidation of NH_4^+ to NO_3^- is coupled to the fixation of carbon by chemoautotrophic bacteria in the genera *Nitrobacter* and *Nitrosomas*. Because NO_3^- ions have high mobility and are less incorporated by plants, they were probably affected more by the regular occurrence of rainfall and availability of dew than by the presence of biological crusts. Comparison of C/N ratios of soil and relative abundance and activity of N fixing and nonfixing microbes among the three classes of crust at our site is necessary to more fully interpret our results [20].

In our study, N fixation occurred throughout the growing season and fluctuations did not follow any appreciable changes in temperature and moisture. However, no net accumulation of N in soil occurred. In regions where rainfall dominates during warm periods, most N fixed and released by crusts is likely to occur when N leaching is also greatest. In these areas, greater losses of N relative to N inputs is likely, and produce smaller transfer of newly fixed N to plants or microbial biomass [3]. Certainly, denitrification processes could simultaneously compete with plants and microbes for the newly released N [3]. However, this is unlikely given the low pH of soils in this study. Furthermore, we were unable to detect denitrifying bacteria using a most probable number method [31]. However, we cannot exclude the possibility of denitrification occurring because we sampled once in May and not throughout the growing season as we did for N fixation and leaching. Decoupling of denitrification and N fixation in time has been reported for biological crusts in some desert ecosystems. For example, most N inputs from living soil crusts of southeastern Utah occur when precipitation occurs in autumn through spring, and is minimal in summer when temperatures are hot [3]. Summer temperatures have potential to have high denitrification rates, but

N_2O fluxes are minimal because most of the N fixed is consumed by plants and microbes from fall until summer.

To our current knowledge, this is the first study to determine the total recovery or extraction efficiency of the IER types chosen for this study to access very low N available in sandy soils. Although Giblin *et al.* [18] tested ion exchange IER bags in low-N ecosystems, it is impossible to compare their study with ours because different IER were employed. Furthermore, most experiments test the efficacy of IER using greater concentrations of N than those used in our study. For example, Kjonaas [27] added approximately 5 μmol of either NO_3^- or NH_4^+ per gram of strong acid IR-120 to verify the extraction efficiency of different strengths of KCl solutions. Our greatest concentration (1.33 μg g^{-1}) was equivalent to 0.14 μmol N per gram of IER. NH_4^+ ions compete with potassium ions for the IER exchangeable sites because they have similar affinities. The exchange reaction is an equilibrium reaction, and when the selectivity of two ions is similar, the concentration of the two ions affects the relative quantity of ions on the IER and in solution [28]. Therefore, it is obvious that a greater load of N facilitates adsorption, whereas small N amounts are unfavorable to compete for IER exchange sites. Thus, a decline in IER recovery as water volume increased may simply be a result of decreased concentration.

In conclusion, areas with varying types of biological soil crust in northwestern Ohio contribute similarly to N fixation. Lichen crusts may accelerate losses of NH_4^+ through leaching, but may retard loss of NO_3^- compared to moss crusts and bare soil. N inputs by biological soil crusts in northwestern Ohio are comparable to those in arid regions. However, Ohio crusts contribute far less N than atmospheric deposition. In addition to regulating input and losses of N, biological soils crusts at our study site moderate fluctuations in soil climate, in part by reducing evaporation and absorbing more solar radiation with their dark pigments.

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