The effect of biological soil crusts on throughput of rainwater and N into Lake Michigan sand dune soils

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Abstract

Biological soil crusts composed of cyanobacteria, green algae, bryophytes, and lichens colonize soils in arid and semiarid ecosystems worldwide and are responsible for significant N input to the soils of these ecosystems. Soil crusts also colonize active sand dunes in more humid regions, but studies of structure and function of such sand dune crusts are lacking. We identified the cyanobacterial, algal, and bryophytic constituents and N production and leachates of biological soil crusts that colonize beach dunes at the Indiana Dunes National Lakeshore along southern Lake Michigan in Indiana, USA. To determine the role of these crusts in this system, we conducted a greenhouse experiment in which intact soil cores with biological crusts were subjected to artificial rainfall over a full growing season. The volume and N content of leachate from the cores were quantified in relation to degree of crust development, taxonomic composition, rainfall volume and intensity, light intensity, and the presence of plant litter. Net N throughput significantly exceeded N inputs to cores in rainwater. Net N outputs from crusts to subsurface soil ranged from 0. 01 to 0.19 g NH_4^+-N m^{-2} yr^{-1} and 0.01 to 0.61 g NO_3^-N m^{-2} yr^{-1}. Thus, total inorganic N inputs associated with biological soil crusts ranged from 0.02 g N m^{-2} yr^{-1} to 0.8 g N m^{-2} yr^{-1}. High volume (≥ 2 cm) rainfall resulted in more N leaching than low volume events, and plant litter added over the surface of crusted soil cores significantly increased the amount of N in leachate. Exploratory path analysis revealed direct and indirect linkages among environmental factors, crust development, and crust composition in regulating the throughput of H_2O and N from these intact soil cores. Biological soil crusts at this site, combined with other properties of the soil surface, substantially increase N inputs to this water- and nutrient-limited sand dune ecosystem.

Introduction

Biological soil crusts influence the physical and chemical properties of soils in arid and semiarid ecosystems by affecting soil water retention and reducing wind and water erosion (Belnap and Gillette, 1998), producing chelating compounds that bind minerals and plant nutrients (Belnap, 1996), and increasing C and nutrient inputs to vascular plants, soil fauna, and soil microbes (Belnap, 2001; Dodds et al., 1995; Millbank, 1978, 1982; Rogers and Burns, 1994). As a result of the significant photosynthetic and

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N-fixation rates of some soil crusts and the capacity for crust cover to approach 100% in plant interspaces, crusts may contribute substantial C and N input to the root zone (Evans and Johansen, 1999; Evans and Lange, 2001; West, 1990). Vascular plants growing among N-fixing soil crusts show increased N and mineral uptake and enhanced growth (Belnap and Harper, 1995; Harper and Pendleton, 1993; Mayland and MacIntosh, 1966).

The metabolic activity of crust organisms is moisture and nutrient limited (Belnap, 2001; Lange, 2001) and crust activity varies widely depending on the composition and relative abundances of crust constituents and environmental conditions (Belnap, 2001; Lange, 2001; West, 1990). For example, lichen and bryophyte-dominated crusts absorb high volumes of surface water upon rehydration after desiccation (Lange, 2001), whereas crusts dominated by cyanobacteria reduce water throughput to vascular plant rooting zones when their polysaccharide sheaths absorb water and swell during rainfall events (Eldridge et al., 2000; Kidron and Yair, 1997).

In contrast to arid and semiarid ecosystems, our understanding of the role of soil crusts in sand dune systems in more humid regions is limited (but see De Winder et al., 1989 and Smith et al., 2004). Most existing studies of crusts colonizing sand dunes focus primarily on the taxonomy of crust organisms and on the impact of these organisms on water relations. Although such sand dune systems are usually strongly nutrient limited, we know of no research that has quantified the influence of biological soil crusts on nutrient movement through the soil profile in such ecosystems.

The overall purpose of this study was to characterize the biological soil crusts in the coastal sand dune ecosystem of the Indiana Dunes National Lakeshore (IDNL), and to evaluate the influence of these crusts on rainwater and nutrient throughput. The Indiana Dunes ecosystem has been studied extensively from the plant ecological perspective (Cowles, 1899, 1901; Hill, 1896; Lyon, 1927; Olson, 1958), and research done at the Indiana Dunes is best known for its contribution to classical ecological theories of succession (Cowles, 1899). However, the role of biological soil crusts on soil properties and plant community dynamics at this site has never been addressed.

Our specific objectives were to: (1) characterize the species composition of the biological soil crusts at the Miller Dunes region of the IDNL; (2) determine whether crust species composition and/or crust cover affects throughput of water and N through the soil profile; (3) assess the relative roles of rainfall intensity, light intensity, and the overlying litter mass on water and N throughput and; (4) determine whether crust composition and surface soil characteristics interact to influence water and N throughput.

Methods

Description of the study site

The Indiana Dunes National Lakeshore (IDNL) (41°37′ N, 87°05′ W) is a system of active sand dunes on the southern shore of Lake Michigan in northwestern Indiana, USA. Within IDNL, our specific study site was the Miller Dunes, a dune complex on the western end of the Indiana Dunes National Lakeshore. Mean annual temperature of the region is 9.7 °C and mean annual precipitation is 99.2 cm (NOAA, South Bend, IN). The soils of the Indiana Dunes are primarily fine and coarse sand (0.05–2.0 mm diameter) with surface organic matter. This area is not developed or managed, but is subject to both natural disturbances (e.g., storm surges, lightning-caused fires) and human disturbance (e.g., ATV use, arson).

Sampling method and experimental design

In June 2000, 90 soil cores were collected from a population of mixed cyanobacterial, algal, and bryophytic soil crusts growing on a leeward dune in an area of the Miller Dunes free from obvious human or natural disturbance. The area experiences some windblown sand burial. The area over which samples were collected was approximately 50 m^2, and sampling was done to ensure that we captured the full range of cyanobacteria/algal and moss coverage (i.e., we ensured a representative range of crust cover on a scale of 0–100% coverage for both cyanobacteria/algal and moss coverage). The vascular vegetation was dominated by American beachgrass (*Ammophila breviligulata* Fernald) and eastern cottonwood (*Populus deltoides* L.).

Samples were collected to a depth of 7 cm using 5 cm-diameter transparent plastic soil sleeves and returned intact to a greenhouse. A pilot study conducted in 1999 revealed that 7 cm was the optimal depth for obtaining leachate under various rainfall and crust cover regimes, i.e., < 7 cm soil depth did not capture the vascular plant rooting zone, but > 7 cm soil depth resulted in negligible leachate through cores. In the greenhouse, intact crusted soil cores were situated in cylindrical soil sleeves and suspended 8 cm above a greenhouse bench by affixing the cores into holes cut in wax cardboard boxes (Figure 1). To prevent loss of the soil through the bottom of the soil sleeve, two layers of sterilized bridal veil and window screen were affixed to the bottom of the sleeve with rubber bands. This method prevented loss of sand grains but allowed leachate to pass through the sleeves. The 90 cores were distributed randomly on the greenhouse bench and rotated weekly. Mean temperature in the greenhouse over the course of the experiment was 25.2 °C (range: 15.3–48.7 °C) and mean relative humidity was 79% (range: 53–97%).

The treatment design was a $2 \times 2 \times 2$ factorial design with cores randomly assigned to binary light intensity, rainfall intensity, and litter application treatments. At Miller Dunes, crusts develop between beachgrass ramets where they are exposed to full sunlight as well as below beach-

grass clumps where they are shaded. These two light intensities were simulated in the greenhouse by placing 45 of the 90 cores under a shade cloth under which incident photon flux densities (PPFD) were approximately 200 μmol s^{-1} m^{-2}, equal to the average incident PPFD measured under beachgrass ramets in the field. Photon flux densities under full sun in the greenhouse were approximately 1630 μmol s^{-1} m^{-2}, equal to the average incident PPFD at the soil surface in fully exposed sunlight as measured in the field. The shade cloth was not moved during the experiment; thus, cores were rotated weekly within their respective light intensity treatment areas.

To simulate different rainfall intensities that occur in Lake Michigan dune systems, 45 of the crusted soil cores were randomly assigned to receive rainfall simulating frontal (low intensity) storm events while the other 45 received rainfall simulating convective (high intensity) storms (ultimately a labeling error made these numbers 44 and 46, respectively). Simulated rain amount reflected the precise rain amount recorded at the Miller Dunes during the months of April and May 1995 (National Atmospheric Deposition Program 1995); these months were chosen because they reflect a time of highest rainfall and high biological activity. Crusts were allowed to dry between rainfall events on days that no precipitation was recorded in official records.
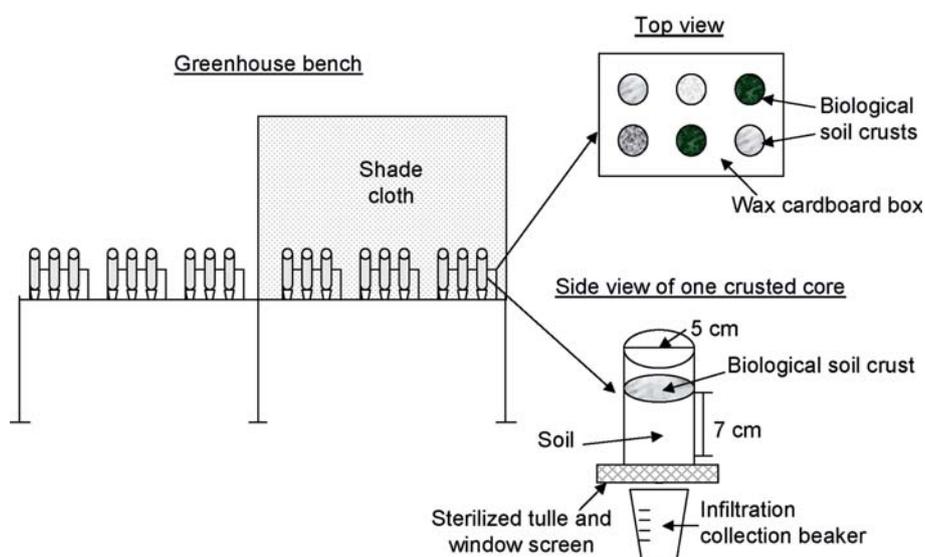

Figure 1. Schematic of the experimental set up for the greenhouse experiment.

During each rainfall event, crusts subjected to convective and frontal storm simulation events received the same volume of rain but at different intensities: convective storms consisted of raining the full rain volume on crusts at one time, and crusts subjected to frontal storms received rain intermittently over the period of 8–15 h. The range of time to simulate frontal storm simulations depended upon how long it took to 'rain' on crusts the given volume recorded for that event, and all simulations began at 7 AM. Rainfall was applied by hand with a pipettor using a Hubbard Brook rainfall recipe that delivers 0.17 mg L^{-1} NH_4-N and NO_3–N (Likens et al., 1996). The simulation experiment began on July 1, 2000 and concluded on September 14, 2000, with 30 total rainfall events over the course of the experiment.

We observed in the field that some crusted areas were overlain with beachgrass litter while others were not. To simulate in the greenhouse the potential physical and biological effects of this litter on water and nutrient leaching, 3 g of field-collected beachgrass litter were placed on the crust surface of 28 soil cores, 14 in shade and 14 in sun. Within each of these light treatments (shade vs. sun), 7 litter-covered cores received low intensity rainfall and 7 received high intensity rainfall. Because adding litter to only 28 cores resulted in an unbalanced treatment design, we manually 'bootstrapped' the 'no litter' treatment level by randomly selecting 28 of the 62 'no litter' data to represent this treatment level, and performed NPMANCOVA to evaluate the effects of light using $n = 28$ for both treatment levels. Random selection of a subset of 28 data and subsequent NPMANCOVA analyses were repeated 10 times for each assessment of the effect of 'no litter' on the response variables; the ranges of pseudo-F and permutation-P values are reported (see 'Data analysis' section and Appendix A).

After each simulated rainfall event, leachate that infiltrated through each soil core was collected in a sterilized beaker (Figure 1). The volume of leachate from each core was measured and the leachate was immediately removed from the greenhouse, stored in an airtight plastic bottle, and frozen pending analysis. NH_4^+ and NO_3^- in each leachate sample were analyzed using colorimetric methods (Keeney and Nelson,

1982). Net NH_4^+ and NO_3^- leached through each core during each rainfall event were calculated by subtracting the amount of N introduced to the cores in rainwater during that particular rainfall event.

Crust species characterization

After the greenhouse experiment was concluded, the relative percent cover of cyanobacteria and mosses in each crust sample was quantified using a transparent 5 cm-diameter circular grid divided into 3×3 mm squares. The grid was placed over each 5 cm-diameter crust, each 3×3 mm square was recorded as cyanobacteria, moss, or open sand, and the percentage of each crust constituent was calculated. This allowed us to obtain continuously distributed estimates of the percent cover of cyanobacteria and mosses in each crust.

After quantifying the relative percent cover of cyanobacteria and mosses in each crust, a representative sample ($n = 10$) of crusts was used to prepare dilution plates to culture and identify cyanobacteria species, using the methods of Flechtner et al. (1998). Dilution plates were incubated for 3 weeks and then a sample of colonies from each plate was examined under a BMAX Olympus light microscope. Each unique species encountered was isolated onto Z8 medium in a small Petri dish for preservation. Although only a small percentage of cyanobacteria and bacteria will be successful in culture (Amman et al., 1995), our initial efforts at characterizing the cyanobacteria of this system serve as a general description of the types of crust constituents in this particular ecosystem. Cyanobacterial taxonomy is based on Anagnostidis and Komarek (1985), Desikachary (1959), and Geitler (1932).

Data analysis

Leachate volume, NH_4^+, and NO_3^- were summed for each crust to achieve a cumulative measurement of each response variable over the growing season, and these cumulative data were used for statistical analyses. The activity of biological soil crusts is highly sensitive to temporal and spatial changes in desiccation and hydration, and the responses of crusts to these changes vary by crust composition and cover. Thus, using cumulative numbers for our data analyses

allowed us to evaluate how crusts contribute to water and N inputs to these soils over the period of one growing season (simulated by us in the greenhouse as cyclical drying and wetting events).

Residual data were non-normally distributed due to high NH_4^+ and NO_3^- leaching during heavy rainfall events and transformation did not improve normality. Further, residual data were not independent, as the outcome of assays collected from any soil core was likely influenced to some degree by earlier rain events. Thus, non-parametric methods were used to assess the influence of light intensity, litter addition, and rainfall intensity on leachate volume and N throughput using % cover of cyanobacteria and % cover of mosses as covariates. Non-parametric Multivariate Analyses of Covariance (NPMANCOVA) were performed on raw data using a statistical software application developed to deal with complex multivariate ecological data, which often lack the distributional properties necessary for the use of traditional statistical methods (Anderson, 2001; McArdle and Anderson, 2001). Specifically, we used the DISTLM v.5 distance-based multivariate analysis developed by Dr. Marti J. Anderson at the University of Auckland, New Zealand, to evaluate the effects of the treatment variables light, litter, and rainfall intensity and their interactions on leachate volume and N throughput, using % cyanobacteria and % mosses as covariates (see Anderson, 2004 for complete description).

Separate NPMANCOVA were performed for each treatment (e.g., light, litter, rainfall) and their interactions (e.g., light×litter, light×rainfall, litter×rainfall), using % cyanobacteria and % mosses as covariates; individual terms in the NPMANCOVA model were tested one at a time (Anderson, 2005). Because replication in the litter treatment levels was imbalanced, bootstrapping was used to balance the design before the NPMANCOVA analyses for the effects of litter, light×litter, and litter×rainfall on the response variables. Specifically, in the analysis of the effects of litter on each response variable, repeated subsets of 28 data were randomly selected from the 62 'no litter' data and 10 separate NPMANCOVA were performed, each using a different random subset of the 'no litter' data. For the analyses of interaction terms that included the effects of litter, each interaction term that included 'yes litter' originally had 14 replications; whereas, each interaction term that included 'no litter' originally had 31 replications. Thus, in the analyses of the interactive effects of light×litter and litter×rainfall, repeated subsets of $n = 14$ from the data in the interaction treatment levels that included 'no litter' (e.g., cores exposed to no litter and shade) were randomly selected and separate NPMANCOVA performed 10 times for each response variable. Ranges of the pseudo-F and permutation-P values are reported for these bootstrapped analyses (see Appendix A).

The NPMANCOVA performed by DISTLM v.5 relies on the computation of distance matrices of raw data to partition the variances among treatment groups, from which a pseudo F-statistic is calculated that is not bound by the assumptions of the F distribution of traditional parametric techniques. A permutation-P value is then obtained after 999–4999 random permutations of data observations (we used 999 permutations) in which continually new F-values are calculated to achieve the distribution of the pseudo F-statistic under a true null hypothesis (see Anderson, 2001 for detailed discussion). Interpretation of the pseudo F-statistic and permutation-P value is the same as for traditional analyses. Statistical significance was determined at $p \leq 0.05$ unless otherwise noted.

We supplemented the results of the NPMANCOVA analysis with exploratory path analysis (Amos 3.51, SmallWaters Corporation, Chicago, IL), a form of structural equation modeling, to further characterize the direct and indirect linkages that could affect the transport of water and N through these soil cores. Exploratory path analysis has been used in this region to characterize landscape and site treatment effects on N transport following fires (Boerner et al., 2000), and has proven superior to traditional multiple regression methods because path analysis allows characterization of both direct and indirect effects of independent variables and their interaction on experimental responses (Arbuckle, 1995).

For path analysis, we first evaluated covariance (colinearlity) among the putative independent variables the influenced the volume and N content of leachate: light intensity, litter cover, rainfall intensity, % cyanobacterial cover, and % moss cover. Pairs of factors that covaried significantly at $p < 0.05$ were retained in the model; all

others were evaluated as causal regression links. Goodness of fit was evaluated by regressing covariance moments implied by the final model against the actual covariances. Although all possible causal links among factors were included in the analytical model, for clarity we included only causal links significant at $p < 0.10$ in the model presented here.

Results

Crust species composition

Thirteen species of cyanobacteria, one species of green algae, and two moss species were present in the crusts (Table 1). Lichens were entirely absent from the 90 soil cores and crusts. Especially abundant in crust samples were the cyanobacteria *Leptolyngbya* cf. *bijugata* Konssinger, *Leptolyngbya tenuissimum* (*hansgirgiana*) [Nägeli] Komarek, *Microcoleus steenstrupii* Boye-Peterson, *Microcoleus* cf. *vaginatus* Vaucher, and *Nostoc commune* [Vaucher] ex Bornet. et Flahaut. The *Nostoc* present in these crusts were heterocystic. Moss spores and protonemata were present,

Table 1. Species found in biological soil crusts from the Miller Dunes region of the Indiana Dunes National Lakeshore, Miller, IN

Cyanobacteria
Leptolyngbya cf. *bijugata* Konssinger
Leptolyngbya cf. *foveolarum* [Rabenhorst] ex Gomont
Leptolyngbya cf. *tenuissimum* (*hansgirgiana*)
[Nägeli] Komarek
Phormidium molle Gom. f. *tenuior* W. et. G.S. West
Microcoleus cf. *paludosus* Kützing
Microcoleus cf. *steenstrupii* Boye-Peterson
Microcoleus cf. *vaginatus* Vaucher
Nostoc commune [Vaucher] ex Bornet. et Flahaut.
Nostoc cf. *punctiforme* Kützing
Tolypothrix campylonemoides Ghose
Scytonema cf. *multiramosum* Gardner
Chroococcus spp. Nägeli
Gleocapsa spp. Kützing
Green algae
Klebsormidium spp.
Mosses
Ceratodon purpureus (Hedw.) Brid.
Sphagnum spp.

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though not abundant, in crusts consisting of primarily cyanobacteria and green algae.

The moss *Ceratodon purpureus* (Hedw.) Brid. was dominant in crusts dominated by bryophytes. *Sphagnum* spp. were less abundant but present within some *C. purpureus*-dominated crusts. No cyanobacteria were present atop or beneath moss gametophytes in crusts consisting of only dense mats of mosses, but most crusts had a mixture of both cyanobacteria and mosses.

To capture the full range of crust cover (0–100%) in our field sampling, six of the 90 cores had no apparent cyanobacterial or bryophytic growth on them when collected. Later dissection of crusts revealed that three of the six uncrusted cores had very sparse *Leptolyngbya* cf. *foveolarum* [Rabenhorst] ex Gomont, *Scytonema* cf. *multiramosum* Gardner, and/or *Microcoleus* colonies approximately 3 mm below the soil surface (i.e., the filaments were covered by 3 mm of windblown sand). We cannot discern whether this growth occurred prior to or during the experiment. These highly motile, filamentous cyanobacterial species colonize uncrusted areas in the field by gliding or oscillating laterally below the soil surface, accounting for subsurface colonization of uncrusted soils. Alternatively, some subsurface cyanobacterial growth may have occurred during our experiment; however, the growth was so minimal as to be undetectable to the naked eye, and was only observable when the crusts were dissected after the experiment was terminated.

Rainwater and N throughput

Over the duration of the experiment, 274 mm of rainwater were applied to the crusts and the cumulative leachate volume from the crusts ranged from 31 to 78 mm. Throughput was greater during high volume (in contrast to high intensity) than low volume storm events, regardless of crust composition and cover (mean throughput in high volume events $\pm$ SE: 20.8 ± 0.9 mL and in low volume events 0.5 ± 0.1 mL, respectively). Taken alone, the proportional cover of cyanobacteria and mosses did not affect cumulative leachate volume (cyanobacteria cover: $r = 0.02$, $p < 0.85$; moss cover, $r = 0.02$, $p < 0.84$).

Litter had no significant impact on the volume of leachate throughput (Appendix A).

Water throughput was, however, significantly affected by light intensity (Appendix A); significantly less rainwater leached through crusts exposed to full sunlight than crusts in shade (Figure 2). Throughput was also affected by rainfall intensity (Appendix A); high intensity storm simulations produced significantly more leachate than low intensity storm simulations (Figure 2).

Overall net N throughput through crusts exceeded N inputs to crusts in rainwater. Total NH_4^+ and NO_3^- inputs to crusts over the growing season were 0.08 μg each, while NH_4^+ and NO_3^- throughput in leachate ranged from 2.8 to 92.7 μg and 15.3 to 1019.7 μg, respectively. Cumulative N throughput through the six crusted cores also exceeded N inputs; however, NH_4^+ and NO_3^- throughput from these crusts was small compared to that from heavily-crusted cores (uncrusted cores, range: 3.7–6.0 μg and cores with developed crusts: 82.1–244.5 μg), and served as an effective benchmark for determining how leachate N was affected by the presence of developed biological soil crusts.

Cumulative N output in leachate was greater during intense storm events than diffuse storm events. Outputs of NH_4^+ and NO_3^- from cores exposed to intense rainfall were 13% and 27% greater, respectively, than that of cores subjected to diffuse rainfall (Figure 3); although differences in NH_4^+ and NO_3^- output between high and low intensity rain storms were not significant (Appendix A).

Output of NH_4^+ from shaded cores was marginally greater than output from cores in full sun (Appendix A), though the difference averaged only 6%, while NO_3^- output was greater from cores in full sun (Figure 4). Neither NH_4^+ nor NO_3^- throughput was significantly affected by light intensity (Appendix A).

The addition of litter to the surface of crusts significantly increased NH_4^+ and NO_3^- output (Appendix A); output of NH_4^+ and NO_3^- from cores with litter cover exceeded that of cores without litter by 41% and 74%, respectively (Figure 5).

Crust composition and throughput

Linear regression indicated that cyanobacterial cover was significantly and negatively, though weakly ($r^2 = 0.018$) correlated with NO_3^- throughput, but was uncorrelated with leachate or NH_4^+ throughput. Moss cover alone had no significant effect on any output parameter.

There was a statistically significant covariance between moss cover and rainfall intensity in determining total leachate volume (Appendix A).
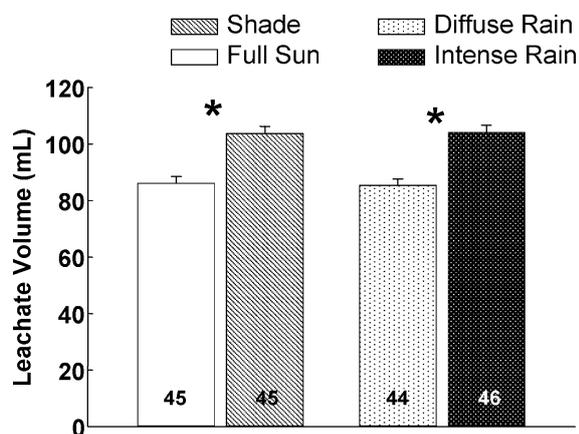

Figure 2. The effects of light intensity (shade vs. full sun) and rainfall intensity (diffuse/frontal vs. intense/convective) on the volume of leachate discharged from intact soil cores. Arithmetic means ± one s.e. are indicated and asterisks denote significant differences between light intensity treatments and between rainfall intensity treatments ($p < 0.05$), as determined by non-parametric multivariate Analysis of Covariance (NPMANCOVA) with % cover cyanobacteria and % cover mosses as covariates. Number in each bar denotes number of samples.

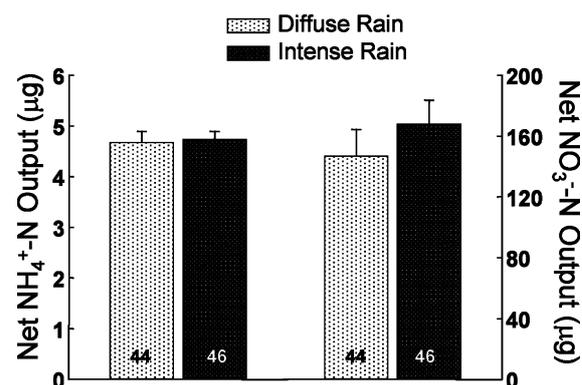Figure 3. Cumulative NH_4^+ (μg) and NO_3^- (μg) output from intact soil cores and biological crusts exposed to diffuse and intense rainfall events. Arithmetic means ± one s.e. are indicated; no significant effect of rainfall intensity on N throughput was detected by NPMANCOVA with % cover cyanobacteria and % cover mosses as covariates ($p > 0.05$). Number in each bar denotes number of samples.

Figure 4. Cumulative NH_4^+ (μg) and NO_3^- (μg) output from intact soil cores and biological crusts exposed to full sun and partial shade. Arithmetic means ± one s.e. are indicated; no significant effect of light intensity on N throughput was detected by NPMANCOVA with % cover cyanobacteria and % cover mosses as covariates ($p > 0.05$). Number in each bar denotes number of samples.
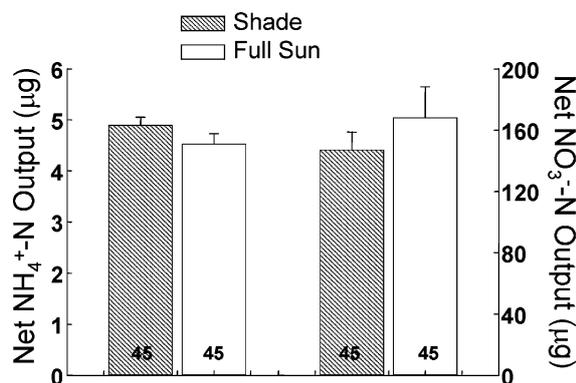
Under high rainfall intensity there was a weak positive relationship between leachate volume and moss cover ($r^2 = 0.065$). There was a similar weak but positive ($r^2 = 0.031$) effect of % moss cover on NO_3^- output, and a weak, negative effect ($r^2 = 0.090$) of the % cover of cyanobacteria on NO_3^- output.

Linkages among factors

Exploratory path analysis produced models for the throughput of water and inorganic N (NH_4^+ + NO_3^-) that accounted for 50% and

19% of the variance among cores, respectively (Figure 6). The two strongest factors regulating the movement of water through these cores were rainfall and light intensity. Leachate volume was positively related to rainfall intensity and negatively related to light intensity. Moss cover and litter cover were not significant factors in determining how much H_2O passed through the cores, and the link with cyanobacterial cover was weak and only marginally significant (Figure 6).

Litter cover and light intensity were both significant, positive effects on the total N output from the cores. In addition, there was a strong indirect effect of light intensity on N output mediated through the effect of light on leachate volume. Neither rainfall intensity nor moss cover contributed significantly to the path model of N output, and, once again, the link between cyanobacterial cover and core response was weak and only marginally significant (Figure 6). There were no statistically significant interactive effects of litter, light intensity, or rainfall intensity treatments on any response variables (Appendix A).

Discussion

The large majority of soil crusts we observed on the weakly consolidated sand dunes at the Indiana Dunes National Lakeshore (IDNL) had thick cyanobacterial layers dominated by species from the N-fixing genera *Leptolyngbya* and *Nostoc*, plus the genus *Microcoleus*, which is typically associated with N-fixing bacteria. De
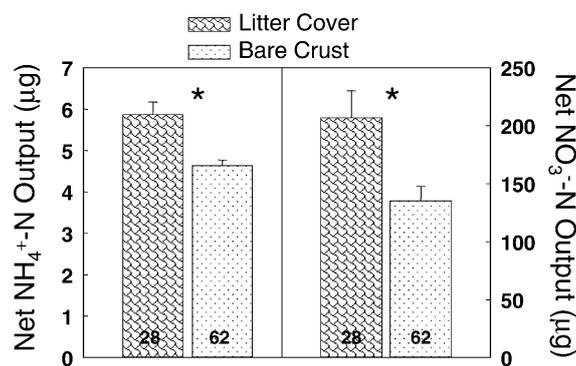
Figure 5. Cumulative NH_4^+–N and NO_3^-–N output from intact soil cores and biological crusts in relation to litter cover condition (i.e., crust covered with beachgrass litter vs. bare soil crust). Arithmetic means ± one s.e. are indicated, and asterisks denote significant differences between little addition treatments ($p < 0.05$), as determined by NPMANCOVA using crust cover as covariates. Number in each bar denotes number of samples.
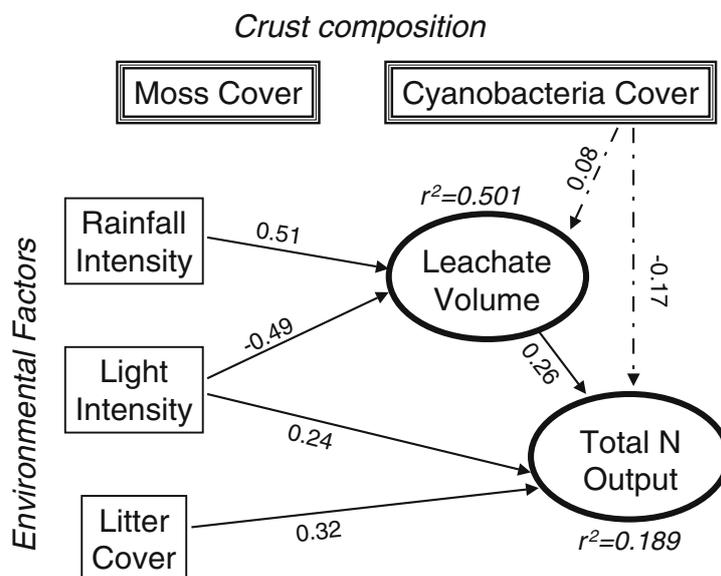
Crust composition

Figure 6. Exploratory path model of the effects of environmental factors and crust composition on the throughput of leachate and N through intact soil cores. Regression links significant at $p < 0.05$ are indicated by solid arrows and those significant at $0.10 > p > 0.05$ are indicated by dashed lines. Regression links not significant at $p < 0.10$ are not illustrated. Goodness of fit for the full model was $r^2 = 0.958$. Standardized regression coefficients are indicated for all links included and the coefficient of determination (r^2) is given for each of the responses ($n = 90$).

Winder et al. (1989) observed a similar suite of cyanobacterial species in blowouts of coastal dunes in the Netherlands; dune crusts at their site were also dominated by motile cyanobacterial species of *Lyngbya*, *Phormidium*, and *Microcoleus*. The motility of these species makes them particularly successful colonizers of active dune systems like the Indiana Dunes and the coastal system studied by De Winder et al. (1989), because the organisms can glide below the soil surface to colonize new areas and can maintain high growth rates at low light levels when they are covered by windblown sand (De Winder et al., 1989). In contrast, Smith et al. (2004) found that biological soils crusts of sand dunes in Cape Cod National Seashore, Massachusetts, USA, were comprised of primarily of green algae (*Klebsormidium/Geminella* sp.) and lacked cyanobacteria.

Identification of soil algae and cyanobacteria can be done in many ways, such as by DGGE fingerprinting (Garcia-Pichel et al., 2003), by direct sequencing from cultures, or by characterization from morphological data. We chose to characterize our cultures using morphological data instead of using molecular methods because

the primary focus of this research was on the overall effects of the biological soil crusts on water and N dynamics; thus, we believed it sufficient to provide a general idea of the types of cyanobacteria present in this system. Despite the limitations of using morphological data, we were able to identify some of the primary constituents of these and other sand dune crusts (De Winder et al., 1989). Molecular methods should be used in future studies at this site to identify the crust constituents that using morphological data may have missed.

Our field observations of crusts suggested that mosses (especially *Ceratodon purpureus*) establish on top of already established cyanobacterial crusts. Soil crusts composed primarily of cyanobacteria are often considered to be early successional crusts, and such crusts often become more diverse with the colonization of mosses and lichens later in crust development (De Winder et al., 1989; Eldridge, 2001; Harper and Marble, 1988). This is envisioned as a facilitative process, as cyanobacteria and associated microbes provide the soil stability, moisture, C, and N necessary for moss and lichen establishment. This facilitative sequence of establishment was

confirmed in our greenhouse experiment, in which we observed moss spores germinating and new gametophytes developing on top of cyanobacterial crusts over the course of the 10–week experiment. De Winder et al. (1989) also observed the same temporal sequence of coastal dune crust colonization, with cyanobacteria and then the green alga *Klebsormidium* (which was also present in our samples) serving as early colonizers that secure sand grains and provide an organic substrate for subsequent bryophyte colonization (De Winder et al., 1989).

One of our primary objectives was to determine how crust composition and cover and various surface soil characteristics influence rainwater throughput in these soils. Light and rainfall intensity had the strongest influence on rainwater throughput. Less rainwater leached through crusts exposed to full sunlight and those exposed to low intensity storm treatments. Full sunlight, as well as the 8–15 h it took to simulate low intensity (frontal) storms, increased evapotranspiration and crust desiccation between rehydration events, increasing the amount of rainwater absorbed by crusts upon rehydration during rainfall events and subsequently decreasing throughput. Further, high intensity convective rainfall had a stronger effect on increasing throughput as the cover of mosses increased. Other studies have also documented greater rainwater throughput through moss-dominated crusts than cyanobacterial crusts (Brotherson and Rushforth, 1983; Ladyman and Muldavin, 1996; Rushforth and Brotherson, 1982). The differential effect of cyanobacteria and mosses on rainwater throughput may be especially pronounced in sandy soils where typical high porosity is blocked by the swelling of cyanobacterial sheaths during rehydration (Verrecchia et al., 1995; Warren, 2001).

The differential effect of cyanobacterial- and moss-dominated crusts on rainwater throughput in this and other studies suggests that the influence of crusts on throughput varies temporally depending upon the phase of crust development (Warren, 2001). Different crust constituents (e.g., green algae, cyanobacteria, and bryophytes and lichens) are present at different phases of crust development, but early and late successional crust stages exist simultaneously in a landscape mosaic (Forman and Godron, 1986). Thus, the influence of crusts on water and nutrient leaching may vary roughly on the spatial scale at which crust composition varies, especially during heavy storm events.

Another objective of this study was to determine how crust and soil characteristics influence nutrient throughput through the soil profile. Net ammonium-N inputs by biological soil crusts at IDNL ranged from 0.01 to 0.19 g NH_4^+-N m^{-2} yr^{-1} and net nitrate-N inputs ranged from 0.01 to 0.61 g NO_3^--N m^{-2} yr^{-1}. Thus, total inorganic N inputs by biological soil crusts at the Miller Dunes ranged from 0.02 to 0.8 g N m^{-2} yr^{-1}. These rates approach or even exceed crust-mediated N inputs recorded in other ecosystems. Biological soil crusts in cold deserts are estimated to fix 10–100 kg N ha^{-1} yr^{-1} (1–10 g N m^{-2} yr^{-1}) (Rychert and Skujins, 1974; West and Skujins, 1977), and crusts contribute 0.7–3.6 kg N ha^{-1} yr^{-1} (0.07–0.36 g N m^{-2} yr^{-1}) to arid Colorado Plateau ecosystems (Jeffries et al., 1992). Estimates of N inputs by crusts in the Sonoran desert and Australia range from 7 to 18 kg N ha^{-1} yr^{-1} (0.7–1.8 g N m^{-2} yr^{-1}) and 1.3 kg N ha^{-1} yr^{-1} (0.13 g N m^{-2} yr^{-1}), respectively (Evans and Johansen, 1999; Rychert et al., 1978). Results from our study suggest that biological soil crusts contribute similar levels of inorganic N to some temperate coastal dune systems as they contribute to arid and semiarid systems throughout the world.

Since considerable fixed N can be lost in the field via denitrification and volatilization, demonstrating that biological soil crusts fix and release N does not necessarily mean that it is all retained in the system N pool for assimilation by plants and soil organisms (Evans and Johansen, 1999; West, 1990). However, in the current study N was measured after it had leached through the soil profile beyond a depth of 7 cm. This suggests that our estimates of crust-mediated N inputs to the IDNL ecosystem reflect available N for utilization by plants and soil organisms after N losses occur. Other authors have drawn similar conclusions. For example, Evans and Ehleringer (1993) used stable isotopes ($\delta^{15}N$) to quantify the relative contributions of atmospheric and soil crust-mediated N to a pinyon-juniper ecosystem in southeastern Utah. They determined that although a substantial amount of crust-fixed inorganic N was lost via denitrification and

volatilization, crust organisms still contributed the majority of total N input to this system.

Where crust cover is well-developed and continuous, the relative contribution of crust-fixed N to the overall ecosystem N budget of IDNL may be substantial. Atmospheric N deposition at the site is approximately 0.3–0.4 g NH_4^+-N m^{-2} yr^{-1} and 1.3–1.4 g NO_3^--N m^{-2} yr^{-1} (National Atmospheric Deposition Program, 2002). Wet N deposition for the state of Indiana is approximately 0.3–0.35 g NH_4^+-N m^{-2} yr^{-1} and 1.2–1.5 g NO_3^--N m^{-2} yr^{-1}, and dry deposition N concentrations are approximately 2.0 μg m^{-3} NH_4^+-N and 4–5 μg m^{-3} NO_3^--N (U.S. EPA 1999). Stable isotope analyses that quantify the relative contributions of atmospheric and crust-mediated N inputs to this dune system would shed light on the relative importance of crust-fixed N to the overall N budget of this ecosystem.

Overall, cumulative NO_3^- throughput through crusts was much greater than NH_4^+ throughput. Nitrifying bacteria associated with crust organisms and organic byproducts (Evans and Lange, 2001; Lange, 2001) may have oxidized NH_4^+ immediately upon its release into the soil profile, causing NO_3^- flushing during heavy rainfall events. Alternatively or in addition, NH_4^+ may have been volatilized and lost as NH_3, which is favored over other processes where N-fixation, extracellular N leaking by cyanobacteria, and soil surface temperatures are high (Haynes and Sherlock, 1986; Schlesinger and Peterjohn, 1991). In this study, we were unable to directly control soil surface temperatures; however, we regulated greenhouse temperature and humidity to be consistent with recorded temperatures and humidity at the IDNL during the time period for which rainfall data were used. Further studies of N dynamics in this system should account for potential discrepancies between soil surface temperatures in a greenhouse and in the field.

Rainfall intensity (frontal vs. convective) did not have a strong effect on N leaching, but rainfall volume (heavy rain days vs. light rain days) significantly increased N throughput (recall that rainfall intensity was an experimental treatment applied to cores; whereas, rainfall volume was the amount of water added to each core during each rainfall event, based on official rainfall data for the site). Overall, net inorganic N flushing through all crusts was remarkably high during heavy storm simulations. Several mechanisms may account for this. Crust-associated bacteria and soil fauna may have been reactivated by rehydration, and their byproducts may account for high C and N mineralization. In addition, extracellular nitrogenous compounds (usually as NH_4^+ and NO_3^-, Belnap, 2001) released by cyanobacteria likely contributed to spikes in N throughput during heavy rainfall events (Millbank, 1982). Also, heavy rainstorms took 8–15 h to complete due to the sheer volume of water 'rained' onto cores; thus, a delay in the onset of maximum nitrogenase activity may have caused heavy N flushing during heavy storm simulations, in contrast to light rain simulations, in which insufficient hydration limited photosynthetic and nitrogenase activity. Although photosynthesis begins almost immediately upon rewetting, N fixation takes longer to reestablish. For example, mixed *Nostoc–Anabaena* and *Nostoc–Scytonema–Microcoleus* crusts take 25–36 h to reach maximum nitrogenase activity after rewetting (Belnap, 2001; Englund, 1978).

Further, crusts dominated by cyanobacteria leached less NO_3^- when rainfall was very heavy, suggesting that cyanobacterial crusts at this site function differently during different rainfall events. Temporary soil anaerobisis resulting from high soil saturation may have activated a denitrifier bacterial community associated with heavy cyanobacterial colonization, causing NO_3^- loss as N_2 and N_2O (Peterjohn and Schlesinger, 1991).

Cyanobacteria- and moss-dominated crusts responded differently to light intensity treatments. Cyanobacteria-dominated cores subjected to full sun had lower NH_4^+ throughput than moss-dominated crusts subjected to full sun. Shading of cores at 200 μmol s^{-1} m^{-2} was probably not responsible for these effects because peak cyanobacterial nitrogenase activity and bryophytic photosynthesis can be reached at light levels as low as 100–200 μmol m^{-2} s^{-1} (Jeanfils and Tack, 1992; Ohki and Fujita, 1988). Instead, high surface temperatures of dark-colored cyanobacterial-dominated crusts exposed to full sun probably exacerbated the negative effects of desiccation on N fixation.

The addition of American beachgrass litter to the soil surface significantly increased N leaching through the soil profile. This effect was not necessarily predictable, as *Ammophila breviligulata*

stems are recalcitrant and showed no noticeable decomposition over the course of the experiment. An active microbial decomposer community associated with beachgrass litter may partly account for increased N throughput where litter was added. In addition, the shade and increased surface humidity provided by litter deposition on cyanobacterial crusts decreases surface soil temperatures and increases moisture levels, thereby increasing N-fixation rates and prolonging N-fixation (Belnap, 2001). The effect of litter addition was especially pronounced as moss cover increased, possibly because surface soil characteristics atop mosses such as lower temperatures and higher humidity may augment microbial community development and activity on decomposing organic matter.

This study is the first to characterize the composition and function of the biological soil crusts at the Indiana Dunes National Lakeshore, and is one of few to describe crusts in active sand dune ecosystems. Biological soil crusts growing among American beachgrass and cottonwood trees at the Miller Dunes region of the Indiana Dunes National Lakeshore do not impede movement of water and nutrients through the soil profile. Instead, the crusts contribute substantial N inputs to this system, particularly under certain environmental and soil surface conditions such as heavy rainfall, shading, and beachgrass litter deposition.

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Appendix A

Results from non-parametric Multivariate Analyses of Covariance to test the effects of light, litter, and rain treatments and their interactions on leachate, NH_4^+, and NO_3^- throughput from soil cores with various levels of biological soil crust cover. Raw data representing the % cover of cyanobacteria and % cover of mosses were used as covariates to each observation. Results from the analyses of the effects of litter on response variables are ranges because these data were bootstrapped (see Methods). The DISTLM v.5 statistical software application was used for the analyses represented here (described in Anderson 2001, 2004). LIGHT = shade vs. sun, RAIN = high vs. low intensity rainfall, and LITTER = litter added vs. no litter added.

Appendix A1. Covariate: MOSS

Source	df	SS	MS	Pseudo-*F*	Permutation *P*
Dependent variable LEACHATE Independent variable LIGHT					
Moss	2	5251.89			
Light	2	5490.06	2745.03	2.78	0.003
Residual	40	39497.58	987.44		
Total	44	51254.21			
Dependent variable NH_4^+ independent variable LIGHT					
Moss	2	7.30			
Light	2	62.75	31.38	0.12	0.62
Residual	40	10409.00	260.23		
Total	44	10456.82			
Dependent variable NO_3^- independent variable LIGHT					
Moss	2	132281.16			
Light	2	1969.06	984.53	0.01	0.87
Residual	40	3297930.87	82448.27		
Total	44	3411510.13			
Dependent variable LEACHATE independent variable RAIN					
Moss	2	2202.31			
Rainfall	2	15594.14	7797.07	8.64	0.001
Residual	41	37004.24	902.54		
Total	45	49483.93			
Dependent variable NH_4^+ independent variable RAIN					
Moss	2	261.63			
Rainfall	2	162.15	81.05	0.32	0.55
Residual	41	10398.49	253.62		
Total	45	10687.58			
Dependent variable NO_3^- independent variable RAIN					
Moss	2	131835.79			
Rainfall	2	81361.62	40680.81	0.52	0.38
Residual	41	3211311.98	78324.68		
Total	45	3388560.21			
Dependent variable LEACHATE independent variable LITTER					
Moss	2	858.48–10439.9			
Litter	2	0–1655.66	0–827.83	0–0.73	0.25–0.99
Residual	23	15037.82–31427.64	653.82–1366.42		
Total	27	25443.5–32557.31			
Dependent variable NH_4^+ independent variable LITTER					
Moss	2	171.28–812.71			
Litter	2	751.71–1879.94	375.86–939.97	0.82–3.18	0.001–0.12 (mean: 0.05)
Residual	23	6723.83–10537.07	292.34–458.13		
Total	27	10633.58–10897.39			

Appendix A1. Continued

Source	df	SS	MS	Pseudo-*F*	Permutation *P*
Dependent variable NO$_3^-$ independent variable LITTER					
Moss	2	149411.12–631346.12			
Litter	2	158459.32–713673.94	79230–356837	0.96–4.48	0.001–0.02
Residual	23	1326751.6–2274209	57685–98878.66		
Total	27	2390718.57–2971957			
Dependent variable LEACHATE independent variable LITTER × LIGHT					
Moss	4	1656.42–23524.02			
Litter × light	3	3246.17–15099.3	1082.06–5033.1	0.25–2.78	0.03–0.99 (mean: 0.37)
Residual	6	9077.48–25784.58	1512.91–4297.43		
Total	13	34169.6–47797.48			
Dependent variable LEACHATE independent variable LIGHT × RAIN					
Moss	4	7485.68			
Light × rain	3	7381.06	2460.35	1.33	0.24
Residual	15	27764.90	1850.99		
Total	22	42631.64			
Dependent variable LEACHATE independent variable LITTER × RAIN					
Moss	4	15845.5–29329.24			
Litter × rain	3	250.44–8300.39	83.48–2766.8	0.02–0.57	0.8–1.0
Residual	6	11994.28–28955.83	1999.05–4825.97		
Total	13	34935.11–56785.19			
Dependent variable NH$_4^+$ independent variable LITTER × LIGHT					
Moss	4	2247.2–5365.2			
Litter × light	3	2240.88–5655.87	746.96–1885.3	1.4–4.91	0.01–0.30 (mean: 0.11)
Residual	6	2304.74–3518.87	384.12–586.48		
Total	13	10704.1–11065.9			
Dependent variable NH$_4^+$ independent variable LIGHT × RAIN					
Moss	4	1535.04			
Light × rain	3	2025.51	675.17	1.20	0.34
Residual	15	8476.59	565.11		
Total	22	12037.14			
Dependent variable NH$_4^+$ independent variable LITTER × RAIN					
Moss	4	2297.1–4980.8			
Litter × rain	3	1798.5–5004.2	599.5–1668.1	0.77–3.72	0.03–0.26 (mean: 0.2)
Residual	6	2462.6–4738.0	410.43–789.8		
Total	13	10828.3–11131.4			
Dependent variable NO$_3^-$ independent variable LITTER × LIGHT					
Moss	4	538995–1644234.5			
Litter × light	3	576008–1311825	176023–437275	0.71–1.43	0.22–0.75
Residual	6	1126393–2492303	176023–437275		
Total	13	3252297–4288950			
Dependent variable NO$_3^-$ independent variable LIGHT × RAIN					
Moss	4	549344.02			
Light × rain	3	409069.69	136356.56	0.50	0.89
Residual	15	4103903.55	273593.57		
Total	22	5062317.26			
Dependent variable NO$_3^-$ independent variable LITTER × RAIN					
Moss	4	925870.2–2450110			
Litter × rain	3	497523–1072517.5	165841–357506	0.74–2.54	0.03–0.65 (mean: 0.34)
Residual	6	680442–1660530.2	113407–276755		
Total	13	3065844–3785379.5			

Appendix A1. Continued

Source	df	SS	MS	Pseudo-*F*	Permutation *P*
A.2. Covariate CYANOBACTERIA					
Dependent variable LEACHATE independent variable LIGHT					
Cyano	2	7060.95			
Light	2	10427.56	5213.78	5.96	0.001
Residual	40	34991.43	874.79		
Total	44	51254.20			
Dependent variable NH_4^+ independent variable LIGHT					
Cyano	2	59.54			
Light	2	86.23	43.11722	0.17	0.69
Residual	40	10420.41	260.51019		
Total	44	10456.82			
Dependent variable NO_3^- independent variable LIGHT					
Cyano	2	146560.60			
Light	2	50628.44	25314.22	0.31	0.64
Residual	40	3293380.74	82334.52		
Total	44	3411510.13			
Dependent variable LEACHATE independent variable RAIN					
Cyano	2	2113.16			
Rainfall	2	6999.83	3499.91	4.25	0.001
Residual	41	33757.59	823.36		
Total	45	49483.93			
Dependent variable NH_4^+ independent variable RAIN					
Cyano	2	324.31			
Rainfall	2	55.04	27.51740	0.11	0.58
Residual	41	10307.43	251.40081		
Total	45	10687.58			
Dependent variable NO_3^- independent variable RAIN					
Cyano	2	53741.06			
Rainfall	2	12586.68	6293.34	0.08	0.67
Residual	41	3288680.92	80211.73		
Total	45	3388560.21			
Dependent variable LEACHATE independent variable LITTER					
Cyano	2	52.33–3326.69			
Litter	2	0–627.89	0–642.62	0–0.52	0.28–0.52
Residual	23	19917.05–31955.7	865.96–1389.38		
Total	27	25443.50–32557.31			
Dependent variable NH_4^+ independent variable LITTER					
Cyano	2	143.48–758.6			
Litter	2	897.62–1807.17	448.81–903.58	1.09–2.47	0.01–0.17 (mean: 0.06)
Residual	23	8385.73–10547.33	364.6–458.58		
Total	27	10633.58–10897.39			
Dependent variable NO_3^- independent variable LITTER					
Cyano	2	52823.26–444814.5			
Litter	2	52287–621808.64	26143.5–310904	0.29–4.64	0.001–0.09 (mean: 0.03)
Residual	23	1541361.49–2435134.4	67015.72–90222.6		
Total	27	2390718.57–2971956.98			
Dependent variable LEACHATE independent variable LITTER × LIGHT					
Cyano	4	7339.7–17623			
Litter × light	3	4423–19589.5	1474.3–6529.8	0.34–2.51	0.05–0.96 (mean: 0.31)
Residual	6	11002.3–25752	1833.7–4292		
Total	13	34169.6–47797.5			

Appendix A1. Continued

Source	df	SS	MS	Pseudo-F	Permutation P
Dependent variable LEACHATE independent variable LIGHT × RAIN					
Cyano	4	9170.23			
Light × rain	3	6193.10	2064.37	1.14	0.38
Residual	15	27268.30	1817.89		
Total	22	42631.64			
Dependent variable LEACHATE independent variable LITTER × RAIN					
Cyano	4	26186–35268			
Litter × rain	3	0–7036.5	0–2345.5	0–0.78	0.63–1.0
Residual	6	8228.3–18171	1371.4–3028.5		
Total	13	34935–56785			
Dependent variable NH_4^+ independent variable LITTER × LIGHT					
Cyano	4	2901.8–7369			
Litter × light	3	920.2–5492	306.7–1830.7	0.43–4.15	0.03–0.81 (mean: 0.45)
Residual	6	2095.6–5909.4	349.3–985		
Total	13	10704–11066			
Dependent variable NH_4^+ independent variable LIGHT × RAIN					
Cyano	4	2174.48			
Light × rain	3	2144.08	714.69	1.39	0.22
Residual	15	7718.58	514.57		
Total	22	12037.14			
Dependent variable NH_4^+ independent variable LITTER × RAIN					
Cyano	4	2746.8–6087			
Litter × rain	3	2865.4–5678	955–1892.7	1.45–9.7	0.01–0.28 (mean: 0.1)
Residual	6	930.3–4427.5	155–738		
Total	13	10890–11131.4			
Dependent variable NO_3^- independent variable LITTER × LIGHT					
Cyano	4	884439–1862397.7			
Litter × light	3	428933.3–1410642.4	142978–470214	0.55–1.7	0.16–0.84
Residual	6	1193592.3–1669875.4	198932–278312.6		
Total	13	3252297.4–4288949.6			
Dependent variable NO_3^- independent variable LIGHT × RAIN					
Cyano	4	607197.29			
Light × rain	3	335113.43	111704.48	0.41	0.95
Residual	15	4120006.54	274667.10		
Total	22	5062317.26			
Dependent variable NO_3^- independent variable LITTER × RAIN					
Cyano	4	1307177–2461422.6			
Litter × rain	3	402021.8–1009449	134007–336483	0.9–2.2	0.05–0.53 (mean: 0.27)
Residual	6	754890–1350923	125815–225154		
Total	13	3065844–3785379.5			