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Surface Disturbance of Cryptobiotic Soil Crusts: Nitrogenase Activity, Chlorophyll Content, and Chlorophyll Degradation

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Cryptobiotic soil crusts are an important component of semiarid and arid ecosystems. An important role of these crusts is the contribution of fixed nitrogen to cold-desert ecosystems. This study examines the residual effects of various intensities and combinations of different surface disturbances (raking, scalping, and tracked vehicles) on nitrogenase activity, chlorophyll content, and chlorophyll degradation in these soil crusts. Nine months after disturbance chlorophyll content of disturbed soils was not statistically different from undisturbed controls, except in the scalped treatments, indicating recovery of this characteristic is fairly quick unless surface material is removed. Differences in chlorophyll degradation among treatments were not statistically significant. However, nitrogenase activity in all treatments showed tremendous reductions, ranging from 77–97%, when compared to the control, indicating this characteristic is slow to recover. Consequently, assessment of crustal recovery from disturbance must include not only visual and biomass characteristics but other phys-

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iological measurements as well. Areas dominated by these crusts should be managed conservatively until the implications of crustal disturbance is better understood.

Keywords arid lands, cryptobiotic soil crusts, cryptogamic crusts, disturbance, microphytic crusts, nitrogen cycles

Cyanobacterial-lichen soil crusts are common in semiarid and arid landscapes of the world, representing over 70% of the living cover in some of these systems. These crusts contribute in many ways to the ecosystems in which they occur, including enhancement of soil surface resistance to water and wind erosion, increased aggregation of soil particles, and increased seedling establishment and survival of some species (Harper & Marble, 1988; Johansen, 1993; Belnap & Gardner, 1993).

In addition, cryptobiotic crusts can also improve the nutrient status of soils. Many cyanobacteria and cyanobacterial components of soil lichens fix atmospheric nitrogen (Belnap, 1992; Skujiņš & Klubek, 1978; Terry & Burns, 1987; West & Skujiņš, 1977). Studies utilizing stable isotopes of nitrogen have demonstrated that nitrogen fixed by cyanobacteria in crusts is available to and used by neighboring vascular plants (Mayland et al., 1966; Mayland & McIntosh, 1966). Plants grown in such crusts show higher concentrations of many essential macronutrients than plants of the same species grown in uncrusted soils (Harper & Pendleton, 1993; Harper & Belnap, unpublished data).

Soil surface disturbances negatively affect the integrity and coverage of cyanobacterial crusts, since the filaments are brittle when dry and both the cyanobacteria and lichens are easily crushed. Recovery rates have been found to depend on the type and extent of disturbance, the availability of nearby inoculation material, and on temperature and moisture regimes that follow disturbance events. Based on biomass and/or visual criteria, estimates for time to unaided recovery of cryptobiotic crusts from disturbance have varied widely, ranging from a few years up to 100 years (Harper & Marble, 1988; Belnap, 1993a; Anderson et al., 1982; Jeffries & Klopatek, 1987; Callison et al., 1985; Cole, 1991). Belnap (1993a) estimated that crusts on sandy soils on the Colorado Plateau require at least 40 years for a full recovery under stable soil conditions.

Up until now, the effects of a disturbance on physiological processes in cryptobiotic crusts have not been examined and the rate of recovery of these processes in natural ecosystems has been neglected. This study examines the effects of different kinds of disturbances on nitrogenase activity, chlorophyll degradation, and chlorophyll content of cyanobacterial-rich soil crusts and the rate of recovery of each parameter from the different kinds of disturbance.

Materials and Methods

Plots were located at Dugway Proving Grounds, 110 km southwest of Salt Lake City, Utah (40° N, 113° W). Site characteristics include an elevation of 1400 m; annual precipitation is 19.4 cm, with 52% falling between April and September; and annual mean temperatures are 10.8°C, with the warmest month (July) averaging 25.7°C. Soils at the study area are lacustrine fine silty loams of the Skumpah Series, a mesic mixed Natrargids with a pH of 8.5–9.0. Quantification of plant and ground cover was done in July 1991, before treatments were applied. These showed that the vegetation was dominated by *Kochia americana* Wats. and *Atriplex confertifolia* (Torr. and Frem.) Wats., with vascular plant cover averaging 5.4% (S.E. 0.3%). Soils were covered by a well-developed cyanobacterial-lichen-moss soil crust, with cover for lichens averaging 24% (S.E. 0.5%) and mosses 7%

(S.E. 0.3%). Of the lichens present, only *Collema* sp. is known to fix nitrogen; it represented approximately 50% of the lichen cover. Of the cyanobacteria present, *Microcoleus vaginatus* (Vauch.) Gom. was dominant, being over 95% of the cyanobacterial biomass. The study area had not been trampled by livestock or subjected to vehicular or foot traffic for over 40 years prior to treatment.

The study area was chosen for uniformity of vegetation, topography, and crustal cover. Treatments were replicated twice and applied randomly within the 3 blocks. Treatments included control plots, shallow raking (surface 2 cm), deep raking (surface 10 cm), scalping away the top 1.0 cm of soil, driving over plots with a tracked vehicle (a 50-ton mobile howitzer with an average ground pressure of an estimated 0.76 kg cm^{-2}) 1, 4, and 10 times, and scalping plus howitzer traffic (4 passes).

Nitrogenase activity and chlorophyll content were analyzed in April 1992 approximately 9 months after the plots were treated. Samples were collected dry. For nitrogen fixation, 20 samples were collected from each treatment. Samples were collected in 2.5-cm diameter transparent, plexiglass tubes that were open at both ends. Tubes were pushed into the soil surface to isolate an intact sample. Samples were immediately corked at both ends and transported to a mobile laboratory in an upright position. The entire sample was wetted with a constant volume of distilled water on arrival at the laboratory and injected with enough acetylene to create a 10% acetylene atmosphere. After injection, the crust samples were incubated for 4 hours at 26°C in a chamber lighted with Chromo50 (5000 K) and cool white fluorescent bulbs. Subsamples (0.25 mL) of the atmosphere from head space above samples in the tubes were analyzed for concentrations of acetylene and ethylene using a Carle FID gas chromatograph equipped with a 244 cm, 8% NaCl on alumina column, using helium as the carrier gas (30 mL min^{-1}). Results are reported in gas chromatograph units, as a conversion to absolute values of *N* requires calibration with ^{15}N.

For chlorophyll measurements, fifteen 1-cm deep samples per treatment were collected dry in 16-mm test tubes and extracted immediately. Techniques outlined in Ronen and Galun (1984) were used. Chlorophyll was extracted from samples with dimethyl sulfoxide (DMSO) in the dark for 45 minutes at 65°C. Samples were then centrifuged, and absorption spectra were measured for the supernatant liquid with a Hewlett-Packard diode array spectrophotometer previously calibrated with a DMSO blank. Absorbance characteristics of both acidified (using 1 *N* HCl) and nonacidified extracts were determined by scanning between 700 and 400 nm. Acidified extracts of the cyanobacterial soil crusts showed a drop at 398 nm (chlorophyll *a*), and a new peak at 362 nm (phaeophytin), and a drop at 665 nm (chlorophyll *a* and phaeophytin). Consequently, readings at 362 nm and 398 nm were used to estimate chlorophyll degradation ratios. Readings at 665 nm (chlorophyll *a*) before and after acidification (with 1 *N* HCl), and at 750 nm for turbidity were used to estimate chlorophyll *a* per unit surface area. Chlorophyll *a* was determined using an equation modified to express pigment content on the basis of surface area:

$$\mu g \text{ chl } a \text{ cm}^{-2} = \frac{26.73(*)\,(v)}{(A)\,(L)}$$

where (*) is the difference in absorbance before and after acidification (of 665 nm–750 nm); (v) is the extract volume in mL; (*A*) is the surface area of the sample in cm^2; and (*L*) is the path length of the spectrophotometer cuvette in cm. This equation accounts for any phaeophytin or turbidity in the sample (Beymer & Klopatek, 1992). Chlorophyll degradation ratios were constructed by dividing readings at 398 nm by readings at 362 nm.

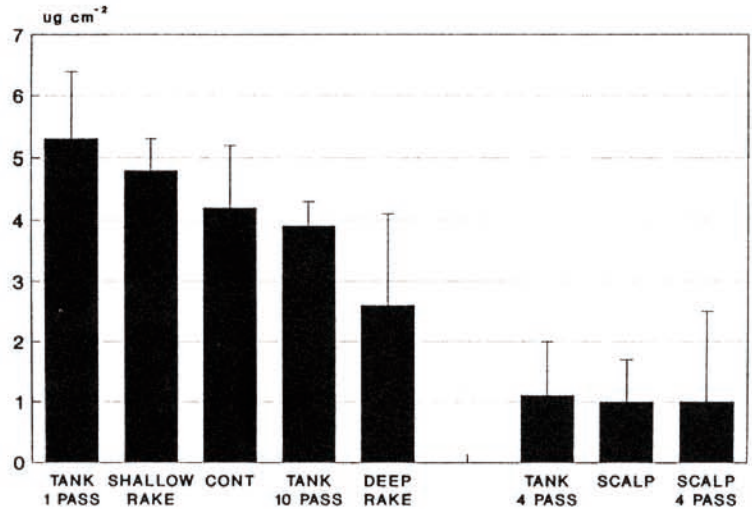

Figure 1. Chlorophyll contents (μg cm^{-2}) for the treatments listed. First group of means differs significantly from the second group ($p < 0.05$). Means within groups do not differ significantly.

Results were analyzed using analysis of variance (ANOVA). Where significant differences were demonstrated by ANOVA, Duncan's multiple range test was used to distinguish treatments from each other. A probability level less than or equal to 0.05 was considered statistically significant.

Results and Discussion

Chlorophyll contents were not significantly different between the undisturbed control and treatments that left the disrupted crusts in place. These included the shallow raked, deep-raked, one-pass, or ten-pass treatments (Figure 1). Treatments that involved the removal of crustal material, scalped and scalped with four passes, did show significantly lower chlorophyll contents than the other treatments and the control. An exception to this was the four-pass treatment, which showed lowered chlorophyll levels as well. It is not clear why this was observed, but it may have been an artifact of sampling. Chlorophyll degradation showed no significant differences among treatments (Figure 2).

The dominant species found in the crusts, *Microcoleus vaginatus*, is fairly tolerant of surface disturbances and burial, being capable of moving up to 5 cm in 24 hours when wetted (Susan Campbell, personal communication). This enables this species to reach photic zones even when covered by water- or wind-borne sediments. Given that this cyanobacterium represented the bulk of the chlorophyll in these crusts, it is not surprising that short-term surface disturbances that left the crushed crusts in place did not significantly reduce chlorophyll levels. Scalped plots, where the biotic crust was removed, would be expected to have lower chlorophyll contents until enough time had passed for the photosynthetic microbial populations to re-establish themselves. Since these organisms are only active when wet, recovery time for the chlorophyll content of soil is heavily dependent on precipitation as well as the extent of disturbance.

Effects of treatments on nitrogenase activity, ranked according to activity level, are shown in Figure 3. The undisturbed control showed significantly higher nitrogenase activity than any treatment. All treatments showed a 77–97% reduction in nitrogenase

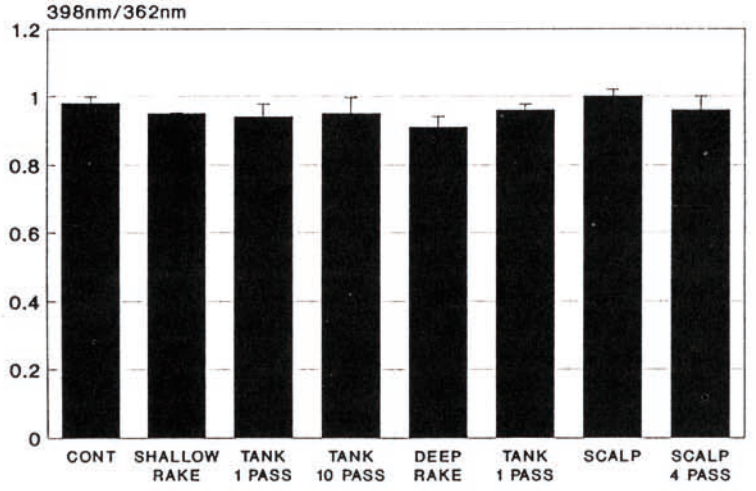

Figure 2. Chlorophyll degradation ratios for the treatments listed. No significant differences were found among mean chlorophyll degradation ratios.

activity due to disturbance of the biotic crust, with a mean reduction of 88%. Treatment means did not differ significantly from each other.

Several nitrogen-fixing lichen and cyanobacterial species were present in these crusts before disturbance. The lichen *Collema* sp. is known to be an active fixer (Belnap, 1992). However, brittle thalli and slow growth rates result in this species being easily extirpated when trampled or buried by sediments. Consequently, most surface disturbances would result in a reduction of this lichen's ability to contribute fixed nitrogen to surrounding soils.

Several known nitrogen-fixing cyanobacteria were present in the undisturbed crusts, including *M. vaginatus, Nostoc* sp., and *Scytonema myochorus.* However, only *M. vagi-*

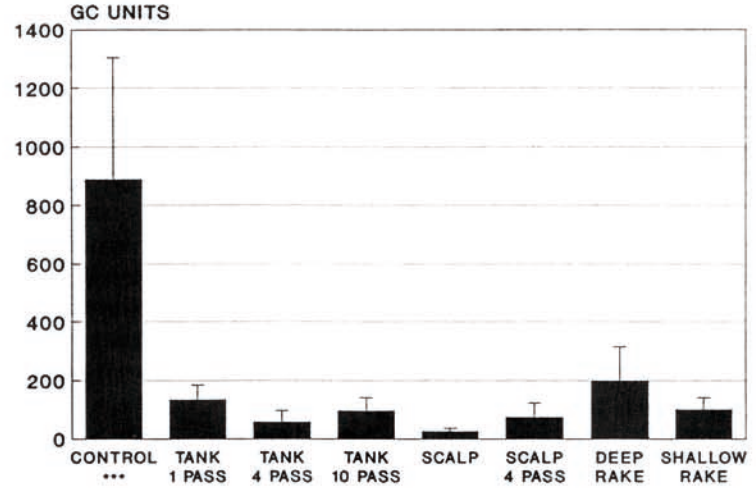

Figure 3. Nitrogenase activity in surface soils that received treatments 9 months earlier. Nitrogenase activity of control was significantly greater than that for all other treatments ($p < 0.02$). Means of treatments did not differ significantly from each other.

Figure 4. SEM micrograph of *Microcoleus vaginatus* (×700). Note the multiple filaments packed inside the extracellular sheath.

natus was present in sufficient numbers to contribute a significant amount of fixed nitrogen. Since nitrogen fixation is an anaerobic process and this cyanobacterium does not have heterocysts (thick-walled cells that exclude oxygen), anaerobic microenvironments must be created in other ways by this species. It is possible that this is accomplished by packing multiple filaments within thick extracellular sheaths (Figure 4) or by packing groups of sheaths together. This packing phenomenon has been shown for several oceanic cyanobacteria, including the morphologically similar species *Microcoleus chthonoplastes* (Paerl, 1985, 1990; Paerl & Bebout, 1988; Paerl & Bland, 1982; Paerl & Prufert, 1987; Paerl et al., 1989a,b, 1991; Pearson et al., 1981). If this is the mechanism employed by *M. vaginatus* for oxygen exclusion, then disruption of tightly packed filaments could create an oxygenated environment and stop nitrogen fixation. Given the extreme brittleness of the sheaths and cyanobacterial mats when dry, virtually any surface disturbance would result in a cessation of nitrogen fixation in this species.

This study demonstrates that recovery time for nitrogenase activity is much longer than for chlorophyll levels and that nitrogenase activity is still greatly suppressed 9 months after the disturbance. This has major implications for studies attempting to assess recovery rates of crusts, where traditional methods have employed visual assessments of chlorophyll levels. In addition, this study shows clearly that nitrogen fixation is not solely determined by the number of cyanobacterial cells present. If nitrogenase activity in *M. vaginatus* is dependent in some way on packing of its filaments, recovery of *N*-fixation activity may be more closely related to the production of sheath material and/or new filaments. Sheath material is produced whenever this cyanobacterium is wetted and its filaments extruded (J. Belnap, personal observation), but there is no information on the rates of production or factors that control it. In addition, nothing is known about factors that control the growth rate of trichomes or what determines the number of trichomes per sheath. It has been noted that the number of filaments per sheath tends to be higher in gypsiferous and limestone soils, as well as in sandy soils that have not been subjected to trampling by livestock or humans (Belnap, personal observation).

This study raises many questions critical to management of semiarid landscapes. These ecosystems naturally maintain little nitrogen in readily available forms, and any activities that adversely affect availability of this often-limiting element may affect the productivity and long-term health of all living components of the system. Research is needed to understand the mechanisms controlling nitrogenase activity in cryptobiotic crusts and how mechanical disturbance affects that activity. With such information available, managers could control actions that damage cryptobiotic crusts and implement actions to hasten their recovery.

References

Anderson, D. C., K. T. Harper, and S. R. Rushforth. 1982. Recovery of cryptogamic soil crusts from grazing on Utah winter ranges. *Journal of Range Management* 35:355–359.

Belnap, J. 1992. Effects of wet and dry pollutants on the physiology and elemental accumulation of cryptogamic crusts and selected rock lichens across the Colorado Plateau. *Final Report, Department of the Interior, Canyonlands National Park.* Moab, Utah.

Belnap, J. 1993a. Recovery rates of cryptobiotic soil crusts: assessment of artificial inoculants and methods of evaluation. *Great Basin Naturalist* 53:89–95.

Belnap, J., and J. S. Gardner. 1993. Soil microstructure of the Colorado Plateau: the role of the cyanobacterium *Microcoleus vaginatus. Great Basin Naturalist* 53:40–47.

Callison, J., J. D. Brotherson, and J. E. Bowns. 1985. The effects of fire on the blackbrush (*Coleogyne ramosissima*) community of southwest Utah. *Journal of Range Management* 38: 535–538.

Cole, D. N. 1991. Trampling disturbance and recovery of cryptogamic soil crusts in Grand Canyon National Park. *Great Basin Naturalist* 50:321–325.

Harper, K. T., and J. R. Marble. 1988. A role for nonvascular plants in management of arid and semiarid rangeland, pp. 135–169, in P. T. Tueller, ed., *Vegetation Science Applications for Rangeland Analysis and Management.* Kluwer Academic Publishers, Dordrecht, Netherlands.

Harper, K. T., and R. L. Pendleton. 1993. Cyanobacteria and cyanolichens: Can they enhance availability of essential minerals for higher plants? *Great Basin Naturalist* 53:59–72.

Jeffries, D. L., and J. M. Klopatek. 1987. Effects of grazing on the vegetation of the blackbrush association. *Journal of Range Management* 40:390–392.

Johansen, J. R. 1993. Cryptogamic crusts of semiarid and arid lands of North America. *Journal of Phycology* 29:140–147.

Mayland, H. F., and T. H. McIntosh. 1966. Availability of biologically fixed atmosphere nitrogen-15 to higher plants. *Nature* 209:421–422.

Mayland, H. F., T. H. McIntosh, and W. H. Fuller. 1966. Fixation of isotopic nitrogen in a semi-arid soil by algal crust organisms. *Soil Science Society of America Proceedings* 30:56–60.

Paerl H. W. 1985. Microzone formation: its role in the enhancement of aquatic N_2 fixation. *Limnology and Oceanography* 30:1246–1252.

Paerl H. W. 1990. Physiological ecology and regulation of N_2 fixation in natural waters. *Advances in Microbial Ecology* 11:305–344.

Paerl H. W., and B. M. Bebout. 1988. Direct measurement of O_2-depleted microzones in marine *Oscillatoria*: relation to N_2 fixation. *Science* 241:442–445.

Paerl H. W., and P. T. Bland. 1982. Localized tetrazolium reduction in relation to N_2 fixation, and H_2 uptake in aquatic filamentous cyanobacteria. *Applied and Environmental Microbiology* 43:218–226.

Paerl H. W., and L. E. Prufert. 1987. Oxygen-poor microzones as potential sites of microbial N_2 fixation in nitrogen-depleted aerobic marine waters. *Applied and Environmental Microbiology* 53:1078–1087.

Paerl H. W., B. M. Bebout, and L. E. Prufert. 1989a. Bacterial associations with marine *Oscillatoria* sp. (*Trichodesmium* sp.) populations: ecophysiological implications. *Journal of Phycology* 25:773–784.

Paerl H. W., J. C. Priscu, and D. L. Brawner. 1989b. Immunochemical localization of nitrogenase in marine *Trichodesmium* aggregates: relationship to N_2 fixation potential. *Applied and Environmental Microbiology* 55:2965–2975.

Paerl H. W., L. E. Prufert, and W. W. Ambrose. 1991. Contemporaneous N_2 fixation and oxygenic photosynthesis in the nonheterocystous mat-forming cyanobacterium *Lyngbya asetuarii*. *Applied and Environmental Microbiology* 57:3086–3092.

Pearson H. W., G. Malin, and R. Howsley. 1981. Physiological studies on in vitro nitrogenase activity by axenic cultures of *Microcoleus chthonoplastes*. *British Phycological Journal* 16: 139–143.

Ronen, R., and M. Galun. 1984. Pigment extractions from lichens with dimethyl sulfoxide (DMSO) and estimation of chlorophyll degradation. *Environmental and Experimental Botany* 24:239–245.

Skujiņš, J., and B. Klubek. 1978. Nitrogen fixation and denitrification in arid soil cryptogamic crust microenvironments, pp. 543–552, in W. E. Krumbein, ed., *Environmental Biogeochemistry and Geomicrobiology*, Vol. 2. Ann Arbor Publishers, Ann Arbor, Michigan.

Terry, R. E., and S. J. Burns. 1987. Nitrogen fixation in cryptogamic soils crusts as affected by disturbance, pp. 369–372, in: *Proceedings, Pinyon-juniper Conference. USDA Forest Service General Technical Report INT-215*. Intermountain Research Station, Ogden, UT.

West, N. E., and J. Skujiņš. 1977. The nitrogen cycle in North American cold-winter semi-desert ecosystems. *Oecologia Plantarum* 12:45–53.