# 19 Factors Influencing Nitrogen Fixation and Nitrogen Release in Biological Soil Crusts

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## 19.1 Introduction

Nitrogen (N) occurs in the atmosphere as $N_2$, a form that is not useable by vascular plants. $N_2$ must first be "fixed", or reduced, to ammonia ($NH_4^+$) by prokaryotic organisms such as eubacteria and cyanobacteria. Thus, an important feature of the cyanobacteria and cyanolichens in soil crusts is their ability to fix atmospheric N. As this fixation is an anaerobic process, most cyanobacterial fixation takes place in heterocysts, which are specialized, thick-walled cells with enhanced respiration that lack oxygen-producing photosystem II (Paerl 1990). Heterocystic genera commonly occurring in soil crusts include *Anabaena, Calothrix, Cylindrospermum, Dicothrix, Hapalosiphon, Nodularia, Nostoc, Plectonema, Schizothrix*, and *Scytonema* (Harper and Marble 1988). Nitrogen fixation has also been demonstrated in nonheterocystous soil genera such as *Lyngbya, Microcoleus, Oscillatoria, Phormidium,* and *Tolypothrix* (Rogers and Gallon 1988; Belnap 1996), although this may be a result of associated bacteria (Steppe et al. 1996). Nonheterocystic species can exclude oxygen in several ways: (1) behaviorally by clumping; (2) spatially or chemically within a cell; (3) temporally, by fixing at night when no oxygen is being evolved by photosynthesis; or (4) through a combination of these (Paerl 1978; Rogers and Gallon 1988; Paerl 1990). Bacteria associated with cyanobacteria may also contribute to N inputs by scavenging oxygen (thus creating anaerobic microzones for the cyanobacteria) or by fixing N themselves. This has been demonstrated for *Microcoleus vaginatus* isolated from soil crusts (Steppe et al. 1996). Soil lichens with cyanobacterial photobionts also fix N. Common N-fixing soil lichens include *Nostoc*-containing *Collema* spp. and *Peltigera* spp. and *Scytonema*-containing *Heppia* spp. Cyanobacteria also live as epiphytes on soil mosses and phycolichens; thus, this consortium of organisms can show fixation activity (Peters et al. 1986).

N fixation activity is generally measured as $^{15}N_2$ incorporation or by the acetylene ($C_2H_2$) reduction assay (ARA). As $^{15}N_2$ uptake is a direct measure of N fixation, it is the most reliable method. However, it is costly and requires

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highly controlled conditions; thus, most studies use ARA. In the presence of $C_2H_2$, the nitrogenase enzyme produces $C_2H_4$ (ethylene); thus, $C_2H_4$ production is a measure of nitrogenase enzyme activity (NA). Because NA is so widely used as a surrogate for nitrogen fixation, these terms will be used synonymously in this chapter.

There are some problems associated with ARA. $C_2H_2$ can affect physiological functioning of the organism, especially when exposure lasts longer than 6 h (David and Fay 1977). The biggest issue is that conversion of ARA data to absolute amounts of $N_2$ fixed requires calibration by $^{15}N_2$, which is seldom done. This is essential, as reported conversion rates in the literature for the $C_2H_4$:$N_2$ ratio vary from 2 to 56, while conversion ratios for naturally-occurring soil cyanobacteria generally range from 1.9 to 6.1 (Potts 1984). However, two recent studies indicate that conversion ratios can be much lower and also can be seasonally variable. S. Phillips and J. Belnap (unpubl. data) found that desert *Nostoc commune* sheets had an average conversion ratio of 0.31. Liengen (1999) reported conversion ratios varied with environmental conditions for both *Nostoc* sheets (0.11–0.4) and free-living soil cyanobacteria (0.02–0.07) in the Arctic. He also analyzed *Anabaena* in culture and obtained a ratio close to 4. These studies may explain why reported rates are so variable, as many studies have used cultured specimens or aquatic species, while actual rates in soils may be much lower. In addition, environmental conditions may lead to large variations in conversion rates. If these more recent results apply to other ecosystems or soil cyanobacterial species, N fixation rates may be currently underestimated by an order of magnitude. This matter clearly needs additional investigation.

## 19.2 Environmental Controls of Nitrogen Fixation

### 19.2.1 Importance of Preexisting Conditions

Many studies have addressed the effects of different environmental factors on N fixation in soil cyanobacteria and cyanolichens. Because past conditions influence current fixation activity through the amount of ATP, reductants, nitrogenase enzyme, and N compounds present in the cyanobacteria, it is essential that past environmental conditions be taken into account when measuring current activity. In light of what we now know about the effects of preexperimental conditions, the seeming contradiction between many data sets can be resolved. Unfortunately, this also makes many data sets unuseable, as preexperimental condition of the organisms was not taken into account by researchers.

The importance of pre-experimental environments on N fixation is well-illustrated by a recent study in southeastern Utah. NA was measured bi-weekly over a 2-year period in *Microcoleus* crust, *Nostoc-Scytonema-Microcoleus* crust, and *Collema* crust (Fig. 19.1). Under standard conditions, specimens in all three crust types showed no or low NA if no rain had fallen within 3 days previous to collection. If rain had fallen within 3 days of collection, NA values were strongly related ($r^2$=0.93) with the average air temperature of the previous 3 days if air temperatures were between 1 and 26 °C (see temperature Sect. 19.2.4). However, there were some exceptions to this pattern, as discussed in the figure legend.

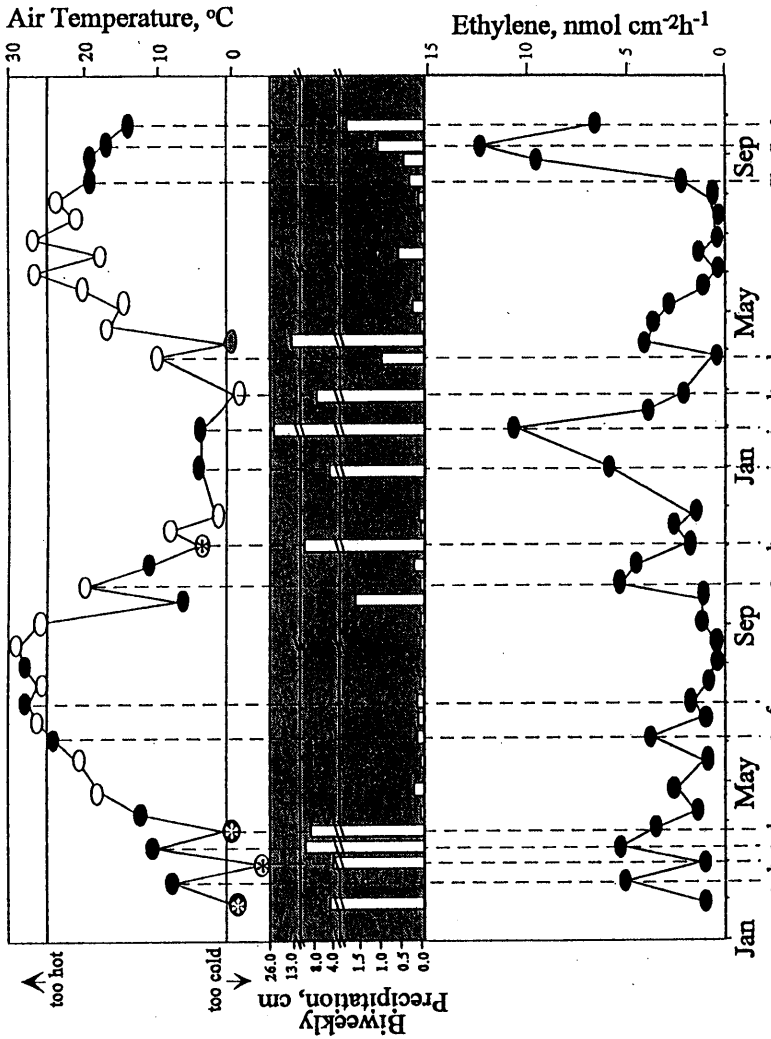
### 19.2.2 Photosynthesis, Carbon Storage, and Dark Fixation

*Photosynthesis/Carbon Storage.* N fixation depends on photosynthesis to provide ATP for energy and carbon compounds as electron donors (Paul and Clark 1996). Consequently, both the duration and rate of N fixation depend on past and current conditions that influence cyanobacterial carbon balance, such as moisture, temperature, light intensity, and supply of assimilates. In the laboratory, exposure to higher light intensity and higher carbon fixation rates before NA assays correlates with higher light and dark N fixation rates in soil crusts (Jones 1977b). Microprobes show incorporation of $^{15}N$ is correlated with gross photosynthetic rates in *Nostoc* (Dodds 1989).

*Dark Fixation.* All soil-crust cyanobacteria capable of light N fixation show dark-fixation activity as well. This includes mixed cyanobacterial crusts and *Nostoc commune* from soils in Hawaii, South Africa (Jones 1977a,b,d), Germany (Potts et al. 1987), Antarctica (Davey and Marchant 1983), the Arctic (O.C. Bliss, pers. comm), China (Scherer et al. 1984), Canada (Coxson and Kershaw 1983a,b), and mixed crusts from Utah (J. Belnap, unpubl.). Rates and duration of dark fixation is limited by endogenous carbon reserves, as polyglucose reserves are mobilized to produce ATP and reductant for N fixation (Millbank 1978). Carbon limitation generally results in dark N fixation being at lower rates than light N fixation in soil crusts from all studied regions (Jones 1977a,b; Rai et al. 1981).

### 19.2.3 Moisture and Rehydration

*Moisture.* Cyanobacteria are physiologically active only when wet; consequently, all N-fixing activities in soil cyanobacteria and cyanolichens are ultimately controlled by moisture (Kershaw 1985; Nash 1996). Moisture can be a result of rainfall, snow melt, dew, or fog. Liquid water is necessary for

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cyanobacterial carbon fixation (see Chap. 18). Because N fixation requires the products of photosynthesis, availability of liquid water ultimately determines its extent.

Moisture levels needed to initiate and optimize N fixation vary widely with species, habitats, and pre-collection conditions. Soil-crust cyanobacteria show NA at values ranging from a water content of 6 % dry weight to total water immersion (Jones 1977d; Kershaw 1985; Belnap et al. 1999). Most soil cyanolichens, including *Collema* species, require a water content of at least 80 % dry weight for initiation of net carbon fixation activity (Lange et al. 1998). NA in *Microcoleus-Collema* soil crusts drops rapidly below soil water potentials of –0.33 kPa, with a 50 % reduction by –1 kPa (Rychert et al. 1978). NA rates at a given moisture content vary within and between species (DuBois and Kapustka 1983; Kershaw 1985). Jones (1977d) found *Nostoc commune* in subtropical soils had maximal NA rates at 22–126 % water content by weight, while Coxson and Kershaw (1983b) showed maximal NA rates at 500 % water content in *Nostoc* from a northern temperate grassland soil. *Nostoc commune* from Mongolian soils had the highest NA rates when totally saturated (Belnap et al. 1999).

As noted above, pre-collection conditions strongly influence NA levels at a given moisture content, presumably by affecting ATP and carbon stores (Kershaw 1985). Constant hydration can also affect nitrogen-fixation rates,

**Fig. 19.1.** Biweekly measurement of acetylene reduction assay (ARA) for 2 consecutive years in field-collected *Collema* from southeastern Utah, measured in the lab under standard conditions (fully moistened, light, 25 °C). *Top panel* Average air temperatures for the sampling date. Samples were collected in early morning. Sample dates with rainfall within 3 days of sample collection are indicated by circle color: *dark* rain; *gray* snow; *clear* none; * snowmelt that added water to soils; *middle panel* Amount of precipitation. Precipitation was measured 10 miles from the sample site; thus amounts recorded only estimate those at the study site; *bottom panel* Nitrogenase activity (NA). Samples were collected, moistened, and incubated under light at 25 °C for 4 h. In general, if soils had been moist within 3 days of collection, NA levels were highly correlated with daily average temperature ($r^2$=0.93) unless temperatures were below 1 °C or above 26 °C. The following letters refer to the *vertical lines labeled at the bottom of the figure: a–d* when temperatures are above 1 °C and soils are moist, NA is observed. Even if soils are moist, low air temperatures preclude ARA activity; however, once temperatures increase, ARA activity resumes. Levels are positively correlated with temperature; *e* temperatures are at the maximum for NA. Soils are moist, but this moisture follows a long dry period, and thus NA levels are moderate; *f* air temperatures exceed the maximum, and although soils are moist, no NA is detected; *g* this sample point was anomalous, as no soil moisture was recorded, but moderate NA were still observed; *h* low temperatures and soil moisture result in low NA levels; *i–k* snow melts, and NA levels soar until air temperatures get too low; *l* in spite of optimal air temperature, lack of moisture precludes NA; *m–o* rain after a long dry period initiates NA. As soils continue to receive moisture, NA increases, although air temperatures are similar. As temperatures decrease, so does NA

with NA rates lower in soil *Nostoc commune* kept continuously hydrated (Jones 1989). Massive glucose efflux was shown in lichens kept continuously hydrated, which could lead to depletion of energy reserves necessary for N fixation (Kershaw 1985). Thus, long periods of high soil moisture may reduce overall N fixation of soil crusts, such as in subtropical or arctic regions. Crusts may also fix less N in ecosystems where dense plant litter keeps organisms wet for extended periods of time. Alternation between wet and dry soils, as occurs in most deserts, may lead to greater N fixation per unit "moist time" than occurs in ecosystems with constantly hydrated soils. However, no study has tested this possibility.

*Rehydration.* Respiration and photosynthesis in cyanobacteria begin almost immediately upon wetting (Chap.18). However, there is generally a lag time between wetting and the initiation of N fixation, with a further lag time before maximal fixation rates are reached. Lag time length is species-dependent, and is also influenced by past and current environmental conditions. There are few data on lag times for initiation of N fixation in crustal species. Free-living and lichenized *Nostoc,* desiccated for a few days, takes minutes to 1 h to initiate NA activity (Kershaw 1985; Dodds et al. 1995). NA in *Scytonema* soil crust begins within 10 min of wetting (Rychert and Skujins 1974; Potts 1980; Jeffries et al. 1992). However, initial NA rates are generally extremely low.

Time to reach maximal fixation rates after rehydration varies widely. Comparison between reported studies must be done with caution, as many authors do not report pre-collection conditions. At 25 °C, a *Nostoc-Anabaena* soil crust showed maximum NA 36 h after being wetted, while Englund (1978) showed maximal NA in a *Nostoc-Scytonema-Microcoleus* crust was reached 25 h after wetting. *Nostoc* has been reported to reach maximum NA at 1–5 h (Jones 1977d; Fritz-Sheridan 1988; Dodds et al. 1995). *Tolypothrix* crusts showed maximal NA rates 6 h after wetting (Fritz-Sheridan 1988). However, none of these studies accounted for potential increases in cyanobacterial biomass.

The longer crustal organisms are desiccated, the more time is required for N fixation to both begin and to reach maximal rates after rewetting. Dodds et al. (1995) reported *Nostoc* samples desiccated for a few days reached maximal NA rates 3 h after hydration, while *Nostoc* desiccated for several months took 8–12 h to reach maximal rates (Nash 1996). Jeffries et al. (1992) found that *Microcoleus* and *Scytonema* crusts required 4–6 days of light and hydration after 6 months' desiccation for maximal NA rates. Scherer et al. (1984) showed that after 2 years of desiccation, *Nostoc flagelliforme* and *N. commune* from northwest China reached maximal NA rates after 120–150 h, while material dried for 2 days required 30–50 h. Again, however, these studies did not measure possible increases in biomass during incubation.

Coxson and Kershaw (1983b) found recovery of maximal NA rates in *Nostoc* after desiccation was also temperature dependent. At 14 and 21 °C, 14 h were needed to recover previously measured maximal rates. Maximum rates were never recovered at 7 °C. Previous exposure to high temperatures also results in a longer time to reach maximal rates. *Scytonema-Microcoleus* crusts exposed to air temperatures of 35 °C for 4 days required wet, light, and cooler conditions (<26 °C) for up to 3 days before maximal NA rates were reached (J. Belnap, unpubl.).

The lag time between wetting and fixation activity probably reflects levels of carbon stores, nitrogenase enzymes, and heterocysts. If there are sufficient carbon stores in the heterocysts, there may be no lag time for initiation of NA. However, if carbon stores are depleted (through prolonged desiccation or having been wet in the dark), photosynthates may first be used to build carbohydrate or energy reserves, and then secondly to service or rebuild the nitrogenase enzyme (Scherer et al. 1984, 1986; Fritz-Sheridan 1988; Dodds et al. 1995; Nash 1996). Consequently, during dry times of the year, or during very dry years, soil crusts may need to be wet longer before N fixation begins, or before reaching optimal N fixation rates, than during wet times of the year.

### 19.2.4 Temperature

Given sufficient moisture and light, N fixation rates are generally controlled by temperature. Most soil cyanobacteria and cyanolichens are capable of N fixation between −5 and 30 °C. Optimum temperatures reported generally range from 20–30 °C for crusts in the Arctic, Antarctica, Scotland, Canada, South Africa, and subalpine regions (Jones 1977b; Stewart et al. 1977; Coxson and Kershaw 1983a; Davey and Marchant 1983; DuBois and Kapustka 1983; Fritz-Sheridan 1988; Lennihan et al. 1994). However, studies of temperature optimum must be interpreted carefully. Constant hydration levels are difficult to maintain in material at higher temperatures and the relationship between tissue moisture and NA is seldom linear; thus, changes in thallus moisture need consideration (Kershaw 1985). Air, and not thallus, temperatures are often reported; however, thallus temperatures can be 15–25 °C warmer than air temperatures on sunny days, while close to the same temperature as air on cloudy days (Belnap 1995). Thus, results can be difficult to interpret if weather conditions are not reported. In addition, Coxson and Kershaw (1983b) warn that, although soil *Nostoc* showed an optimum at 35 °C air temperature for several hours, the long-term optimum for this species was 28 °C.

Minimum air temperatures for NA varies. Field measurements of NA in free-living and lichenized *Nostoc* (in *Leptogium*) have been recorded at −7.6 and 0 °C, respectively (Horne 1972; Davey and Marchant 1983). *Stigonema* and *Scytonema* crusts showed no NA at 0 °C (Isichei 1980). Freezing can sub-

stantially affect NA rates. After freezing, recovery of NA is dependent on conditions before and after the freezing event. Freezing has the ability to damage nitrogenase in *Nostoc* (DuBois and Kapustka 1983; Scherer et al. 1984). Low temperatures can reduce photosynthetic rates and thus reduce available ATP and reductant pools, creating a lag time after freezing before N fixation is initiated (Kershaw 1985). When *Nostoc* was frozen for 3 days and then thawed at different temperatures, recovery of NA to prefreeze levels was much faster at 25 and 30 °C when compared to lower temperatures. Light exposure before freezing reduced recovery time, as well as protein synthesis inhibition, indicating the need for de novo nitrogenase production (DuBois and Kapustka 1983; Scherer et al. 1984).

Above a minimum temperature that is somewhat species-dependent, N fixation rates show a strong, positive response to increasing air temperature until an upper limit is reached, after which rates quickly decline. As seen in Fig. 19.1, *Collema* crusts in southeastern Utah showed potential NA values that were strongly related with average air temperatures of the previous 3 days ($r^2$=0.93) if crusts had received precipitation within 3 days prior to collection. With one exception, NA was observed only when air temperatures were between 0 and 26 °C. NA in *Scytonema-Microcoleus* and *Microcoleus* crusts from the same area was related to values obtained for *Collema* crusts ($r^2$=0.86), indicating that similar environmental conditions control NA for different crust types in this region. Similar upper temperature limits are reported for other species from other environments. *Nostoc-Microcoleus-Schizothrix* crust showed higher NA at 20 °C than at 39 °C (Englund 1978). *Stigonema* from Brazil and *Scytonema* from Nigeria showed no NA at 0 °C, maximum NA at 25–30 °C, and a sharp decline above 30 °C. *Scytonema* crusts from Nigeria showed maximal NA at 40 °C, declining to 0 at 45 °C (Isichei 1980). *Nostoc commune-Cylindrospermum* crust from Scotland showed very low NA at 0 °C, reaching maximum NA at 15-25 °C. A slight decline was seen at 35 °C. Subalpine *Tolypothrix* crusts showed NA at 5 °C, with maximum NA at 20–30 °C (Stewart et al. 1977; Fritz-Sheridan 1988). Loftis and Kurtz (1980) reported that NA ceased in mixed cyanobacterial crusts when soil-surface temperatures reached 45–50 °C. The thallus temperature of *Nostoc commune* was always below 38 °C when NA was observed (Coxson and Kershaw 1983b). Because higher temperatures inhibit N fixation, fixation in desert crusts is generally higher during cooler fall, winter, and spring temperatures. Conversely, low temperatures in the polar regions restrict N fixation in soil crusts to the summer season (Horne 1972; Davey and Marchant 1983).

### 19.2.5 Light

Many studies have examined the effects of light on N fixation of cyanobacteria and lichens. Unfortunately, many of these data are not directly comparable, as different light measurement units were used and/or moisture status, temperature, and pre-experimental conditions of organisms were not controlled. For example, pre-experimental conditions can influence distribution and concentrations of photosynthetic and UV-screening pigments, which, in turn, can greatly alter light attenuation and photosynthetic rates in cyanobacterial colonies and lichen tissue (Dodds 1989; Dodds et al. 1995; Garcia-Pichel and Belnap 1996). Light optima can also be expected to vary with soil characteristics and with cyanobacterial distribution within the substrate, colony, or thallus. In spite of these complications, we can draw some conclusions useful for understanding the effects of light on N fixation.

Soil cyanobacteria and cyanolichens generally reach light saturation for maximum NA at relatively low light levels (Fig. 19.2). Below this level, there is

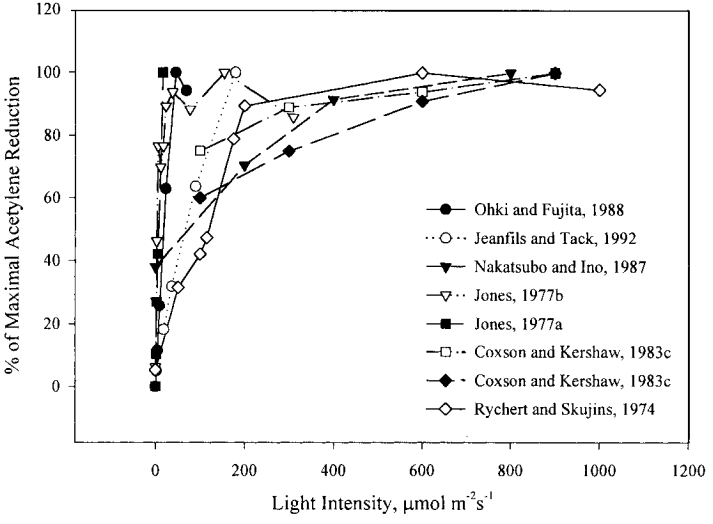


**Fig. 19.2.** The influence of light (PPFD) on nitrogen fixation activity in cyanobacterial soil crusts from the Arctic, Antarctic, tropics, Canada, and the Great Basin (USA). Different light intensity data were converted to photosynthetically active photon flux density using conversion factors for various light sources. However, differences in lamps and sensors make data conversion and comparison of experiments conducted with different light sources imprecise, and errors of +20 % are not uncommon (McCree 1981). As can be seen, optimal nitrogenase activity is obtained at low PPFD, and then maintained throughout the levels measured (Rychert and Skujins 1974; Jones 1977a,b; Coxson and Kershaw 1983c; Nakatsubo and Ino 1987; Ohki and Fujita 1988; Jeanfils and Tack 1992)

a general, but not always linear, correlation between light levels and nitrogen fixation rates, presumably due to the reduced availability of photosynthetic products to support N fixation (Kershaw 1985). Free-living and lichenized *Nostoc* can grow in very low light (see Chap. 16; Dodds et al. 1995; Garcia-Pichel and Belnap 1996). In many studies, *Nostoc* showed an increase in NA up to 100–150 μmol $m^{-2} s^{-1}$ PPFD, after which maximal NA rates are maintained (Jones 1977b; DuBois and Kapustka 1983; Fritz-Sheridan 1988; Lennihan et al. 1994). Other species, including *Calothrix, Stigonema*, and *Scytonema*, also reach maximal NA rates at lower PPFD levels, which are maintained at higher light as well (Griffiths and Gallon 1987; Fritz-Sheridan 1987). Rychert et al. (1978) found that a *Microcoleus-Scytonema-Nostoc-Collema* crust reached optimal NA rates at 200 μmol $m^{-2} s^{-1}$; these rates were sustained at higher light as well. In contrast, two studies have found higher fixation at relatively higher light levels: *Nostoc* showed maximal NA at 400–800 μmol $m^{-2} s^{-1}$ (Nakatsubo and Ino 1987), while Isichei (1980) found no saturation in *Scytonema* crusts from Nigeria, as NA increased up to 34 000 lx (ca. one third maximum sunlight), which was the highest light level tested.

Depression of N fixation by high light has been reported. However, most of these reports are from cyanobacteria in culture, aquatic species, marine mats, or cyanobacteria found on leaf surfaces (Smith 1984; Fernandes and Leganes 1990). Some studies in soil systems are often cited as evidence that high light can limit N fixation (for example, see Reddy and Giddens 1975; Jones 1977c; Rychert et al. 1978; Coxson and Kershaw 1983a). However, high air temperatures (>27 °C), low moisture content of the organisms, and/or failure to account for differential crustal biomass could also explain these results. Any suppression of NA at higher light levels has yet to be conclusively demonstrated.

### 19.2.6 Interaction of Temperature, Moisture, and Light

Based on the above discussion, environmental controls on N fixation are clearly hierarchical. As with photosynthesis (see Chap. 18), the ultimate control on the duration and rate of fixation is the moisture content of the organism. With sufficient hydration, the presence and accessibility of photosynthetic products and nitrogenase enzymes controls fixation activity. This requires access to either intracellular carbon stores (or adequate light to replenish carbon stores). With sufficient hydration and access to carbon stores, temperature then controls fixation rates until carbon stores are exhausted.

This hierarchy is shown clearly by laboratory studies done by Belnap (Fig 19.1), as well as field work done by Coxson and Kershaw (1983c; Fig 19.3). Both studies simultaneously recorded field temperatures, rainfall, thallus moisture, and NA in the soil crusts. Both data sets clearly show NA drops as

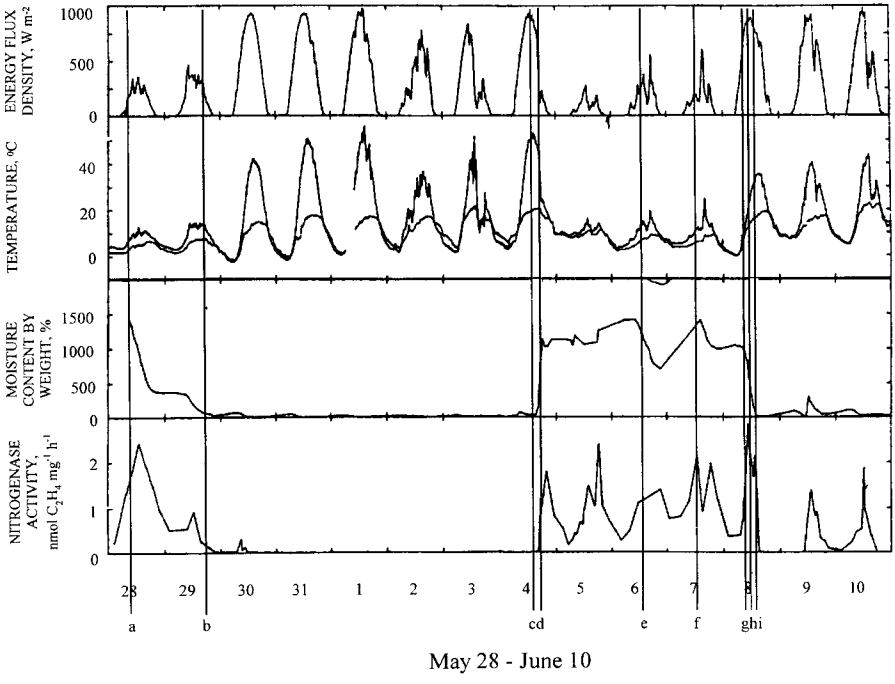


**Fig. 19.3.** Environmental controls on nitrogen fixation measured under field conditions in the Arctic. (After Coxson and Kershaw 1983c). The following interpretation demonstrates how ARA levels are determined in a hierarchical fashion. Dates are May 28 (5/28) through June 10 (6/10). *Top panel* Surface energy flux; *second panel* thalli (*top line*) and air (*bottom line*) temperature; *third panel* moisture content by weight of the soil crust (note smaller-scale *inset* at top); *bottom panel* measured NA. The following letters refer to the *vertical lines in the figure: a* as moisture contents drop (5/28–29), so does ARA, in spite of favorable temperatures and light; *b* from 5/29–6/4, the soil crusts are not wet and so no ARA is observed, although light and temperatures are favorable; *c* on 6/4, crusts are moist. However, temperatures are well above 30 °C, and little ARA is observed; *d* however, as soon as the temperature drops below 30 °C, ARA soars; *e–g* from 6/4–6/8, crusts are moist. Increases in temperatures and light increase ARA values, while drops in temperature and light are followed by drops in ARA, with some lag time noted; *h* on 6/8, crusts experience the most optimal conditions during this experiment (moisture 1000 %, air temperature 25 °C, light 800 W $m^{-2}$); this is reflected in the highest ARA recorded during this time; *i* shortly thereafter, the crusts dry out and ARA stops, in spite of favorable light and temperature

moisture drops, in spite of favorable temperatures and light. With sufficient hydration, rising temperatures initially increase NA values, while drops in temperature and light are followed by drops in NA. At air temperatures above 26–30 °C, little NA is observed. As soon as the temperature drops, NA is resumed. Although this overall pattern is clear in these two data sets, there are

still some anomalous data points that resist explanation, indicating that refinement of these relationships is still needed.

### 19.2.7 Other Factors

*Snow.* Little or no NA has been reported from cyanobacteria or cyanolichens under snow (Englund and Meyerson 1974; Alexander and Kallio 1976; Crittenden and Kershaw 1979), despite the ability of nitrogenase to function unimpaired after thalli had been exposed to temperatures of –40 °C (Kershaw 1985). Laboratory experiments with material collected from under snow show normal NA rates when incubated with light and warmer temperatures, while material kept dark and warm showed no recovery of NA (Huss-Danell 1977; MacFarlane and Kershaw 1980). This would suggest that low light and temperature, resulting in low energy and reductant pools, are responsible for lack of NA under snow cover, not inactivation of the nitrogenase enzyme (Coxson and Kershaw 1983c; Kershaw 1985). Recovery of N fixation after freezing is discussed in Sect. 19.2.4 above.

*Seasonality.* Seasonality in NA rates of temperate soil crusts has been reported by multiple authors, with reported rates being lower during hot and cold periods (summer and winter), and higher in late spring and early fall (Stewart 1967; Johnson 1982; Huss-Danell 1978; Coxson and Kershaw 1983b; DuBois and Kapustka 1983; Kershaw 1985). Summer in temperate regions is generally a time of low moisture and high temperatures, factors that both limit metabolic activity time and lead to inactivation of nitrogen fixation. Low winter temperatures limit both carbon and N fixation. Early fall and late spring, on the other hand, are the times when soil moisture and moderate temperatures most often occur together, and therefore would be when both carbon and N fixation rates are the highest (Coxson and Kershaw 1983b; Kershaw 1985) or greatest cyanobacterial biomass (Jones 1981). Cyanobacterial biomass is greatest after warm (but not hot) wet periods, such as are found in late spring–early summer or early–late fall (Lynn and Cameron 1972; Johansen and Rushforth 1985; Johansen et al. 1993). These studies suggest that there is no endogenous component to nitrogen fixation, but that rates are dictated mostly by different combinations of moisture and temperature (Kershaw 1985).

*Salinity and pH.* There are few data available on the effect of salinity on N fixation in soil cyanobacteria. A study comparing species in saline soils showed that 15 N-fixing species belonging to six genera preferred soils with high electrical conductivity (70 mmhos $cm^{-1}$). Exceptions were *Microcoleus, Phormidium, Nostoc, Tolypothrix, Gloeotrichia,* and *Hapalosiphon,* which

were found in soils with low conductivity (6.5 mmhos $cm^{-1}$; Ali and Sandhu 1972). Crust development was found to be greater on soils with higher electrical conductivity when compared to nearby soils with lower conductivity (62 vs. 39 mmhos $cm^{-1}$ soil; Anderson et al. 1982).

Growth and N fixation in soil cyanobacteria is greatest at pH 7 or above (Brock 1973; Davey and Marchant 1983; DuBois and Kapustka 1983), although some depression of NA has been seen at pH 8–10 (Granhall 1970). Stewart et al. (1977) found NA in soil *Stigonema* increased from low rates at pH 4 to maximal rates at pH 8; a slight decrease was noted at pH 10. Isichei (1980) reported maximal NA in *Scytonema* crusts at pH 6–9, with declines at pH 4, 5, and 10. *Anabaena* and *Nostoc* can fix N down to pH 4, with an optimum at pH 7 or above (Granhall 1981). NA depression at low pH is likely due to reduced photosynthetic capacity (Shapiro 1973) or changes in the nitrogenase enzyme (Stewart et al. 1977). Soil crusts occur in soils with a wide range in pH. Based on the above information, crusts on alkaline soils (dominant in most deserts) are expected to fix more N per unit surface area than crusts on more acidic soils.

*Soil Chemistry.* Soil chemistry has been shown to affect N fixation in cyanobacteria. Most studies have focused on N compounds in the medium regulating NA. Elevated ammonium depresses N fixation, while mixed results have been obtained with elevated nitrate. However, similar results were obtained when NaCl was added, suggesting that a nonspecific salt effect may be partially responsible (Delwiche and Wijler 1956; Reddy and Giddens 1975; Klubek and Skujins 1980).

Lime additions have also been found to enhance NA on pH 6 soils (Reddy and Giddens 1975). However, it is not known whether the stimulation was due to increases in pH or Ca. Phosphorus additions have been shown to stimulate NA in cultures, in pot trials, and in field experiments, perhaps through a stimulation of ATP synthesis (Granhall 1981; Metting 1981; Fritz-Sheridan 1988; Dodds et al. 1995). N fixation in soil *Nostoc* (both free-living and lichenized by *Collema*) is stimulated by low concentrations of As, Ni, Pb, Pd, and Zn. However, longer-term exposure to Cd, Pb, and Zn inhibits fixation (Henriksson and DaSilva 1978). Alexander et al. (1978) report additions of Mb and Co stimulate NA. Boron, Mg, Mo, Va, and Fe are also important for N fixation. However, high levels of Fe, Al, Mn, Cu, Zi, and Hg adversely affect NA (Granhall 1981; Dodds et al. 1995).

*Bacterial Associations.* Bacterial associations may be essential for growth and N fixation in some species of cyanobacteria. Bacteria may scavenge oxygen and thus create anaerobic microzones, replenish $CO_2$, contribute bacterially fixed N, and secrete growth factors and vitamins required by the cyanobacteria (Paerl 1982). Bacteria inside the sheath of *Microcoleus*

*vaginatus* isolated from Utah soil crusts appear to contribute fixed N (Steppe et al. 1996).

*Associations with Mosses and Vascular Plants.* Cyanobacteria can grow epiphytically on soil mosses and lichens. These cyanobacteria may be better protected from desiccation and high temperatures, which can limit N fixation activity. Moss tissue may also protect cyanobacteria from contact with low pH substrates. Mosses both secrete alkaline substances and use the high cation-exchange-capacity of their leaves to buffer the pH of leaf surfaces (Englund 1978; Davey and Marchant 1983; Smith 1984). Light, however, may be limiting, due to shading by the moss tissue (Smith 1984).

Interactions with vascular plants may inhibit or stimulate N fixation in cyanobacteria and cyanolichens. While overhanging plant cover can moderate desiccation and high temperatures and prolong fixation time, leachate from some shrubs may inhibit N fixation (Skujins and Klubek 1978). In addition, as vegetation limits light and soil space, crust cover, and therefore N inputs to the soils, declines (Kapustka and Rice 1978).

## 19.3 Nitrogen Release to the Surrounding Substrate

Extracellular release of nitrogenous compounds occurs in both cyanobacteria and lichens (Stewart 1970; Millbank 1982). It is especially pronounced in N-fixing organisms, which release 5–70 % of N fixed. In cultures of *Nostoc, Scytonema,* and *Anabaena*, the proportion of fixed N that is released is greater in young cultures than in older cultures, indicating that release is not due to cell turnover. Release is greater when pH, temperature, light, $CO_2$, or ionic conditions are not optimal, or during wetting after desication (Henriksson 1957; Jones and Stewart 1969a,b; Millbank 1982; Jeanfils and Tack 1992). Some of the liberated N is reassimilated by the cyanobacteria or lichen, but *Nostoc* has a limited ability to do so (Meeks et al. 1985).

Nitrogen release to the surrounding substrate has been documented in many diverse soil-crust organisms, including *Scytonema* from Nigeria, *Nostoc commune* from New Mexico (Dodds et al. 1995; J. Belnap and F. Garcia-Pichel, unpubl.), *Microcoleus-Nostoc-Scytonema-Collema* crusts from southeastern Utah (F. Garcia-Pichel and J. Belnap, unpubl.) and from northern Utah (Klubek et al. 1978), *Nostoc* soil crusts (Stewart 1967), *Collema tenax* from Europe (Henriksson 1957) and southern Utah (J. Belnap and F. Garcia-Pichel, unpubl.), and *Peltigera canina* (Millbank 1978). N compounds released include small amounts of amide, peptides, and free amino acids, with most as nitrate and ammonium. Released N is readily taken up by surrounding organ-

isms, including vascular plants, fungi, actinomycetes, and bacteria (Stewart 1967; Jones and Stewart 1969a,b; Rogers and Burns 1994).

Many hypotheses have been advanced to explain extracellular release of N. Dodds et al. (1995) suggest that loss by outward diffusion may be unavoidable in low N environments. Millbank (1982) and Potts (1984) consider N release as an unavoidable result of membrane deformation with desiccation and rewetting, as is seen with $^{14}C$, sucrose, fructose, mannitol, glucose, erythritol, glycerol, ribitol, and polyols in cyanobacteria, mosses, and lichens (Farrar 1976; Coxson et al. 1992). Others hypothesize that since N compounds can be toxic, release may be necessary if assimilation, conversion or storage capability in cells is limited (Stewart and Rogers 1977; Bergmann et al. 1992; Silvester et al. 1996). N release may also attract beneficial organisms.

## 19.4 Nitrogen Budgets

As discussed above, ample data demonstrate that biological soil crusts can contribute significant amounts of fixed nitrogen to the ecosystems in which they occur. However, very little is known about the relationship between gaseous N losses and the presence of biological soil crusts. Some studies report that denitrification losses may be significant in arid ecosystems (Peterjohn and Schlesinger 1991) and volatilization rates minimal relative to soil N pools. Klubek et al. (1978) reported volatilization rates were 0.4% of fixed $^{15}N_2$ after weeks of incubation. Evans et al. report low rates in undisturbed *Scytonema-Nostoc-Collema* crusts (Chap. 20). Klubek et al. (1978) found 75–80% of fixed $^{15}N_2$ was lost from dead, decomposing crusts (kept dark and wet for 10 weeks). Because volatilization was extremely low, they concluded the losses must have been from denitrification. $N_2O$ emissions from three sites in the Sonoran Desert (Guilbault and Matthias 1998) showed fluxes ranging from −1.6 to 7.3 ng $N_2O$-N $m^{-2}\,s^{-1}$, with a mean flux of 2.4 $N_2O$-N $m^{-2}\,s^{-1}$. Rates of 0.02 ng $N_2O$-N $m^{-2}\,s^{-1}$ were observed in Great Basin soils (Mummey et al. 1994). However, it is not known if soil crusts were present in these latter two studies. Field measurement of gaseous losses in wetted, living soil crusts of southeastern Utah (K.T. Bradley et al., unpubl.) during fall and spring (when soil temperatures are relatively cool) show quite low denitrification and volatilization rates. $N_2O$ emissions from either *Microcoleus* or *Scytonema-Nostoc-Collema* crusts peaked at 7.1 to 13.9 ng $N_2O$-N $m^{-2}\,s^{-1}$, while $NO_x$ fluxes averaged 0.07 ng $NO_x$-N $m^{-2}\,s^{-1}$. However, summer measurements (when soil temperatures are relatively high, but dry rapidly) showed a relatively high $NO_x$ flux, while $N_2O$ fluxes very low. As discussed previously in this chapter, most N inputs from crusts in this ecosystem occur in fall, winter, and spring, with only minimal fixation occurring during summer. Thus, in

this ecosystem, N inputs and N losses appear decoupled in time. Nitrogen fixed and released appears readily available to plants and microbes from fall until summer (that is, most of the growing season), at which point much of the available N appears lost through volatilization. This would explain why plants growing in crusted soils have higher N than those growing in uncrusted soils, but, at the same time, why N in crusted soils does not accumulate over time.

A similar scenario might be expected in other deserts that receive most of their rain during cool periods. However, regions with rainfall dominant during warm times, or hot deserts where soils are warm most of the year, may be quite different. In these deserts, most N fixed and released by crusts is likely to occur at the same time as N losses are greatest. Thus, in these areas, higher N losses relative to N inputs, and less transfer of newly fixed N to plants and microbial biomass is a likely result, as volatilization and dentrification processes would be competing simultaneously with plants and microbes for the newly released N.

## 19.5 Conclusions

Arid and semiarid soils are generally N-limited and biological soil crusts can often be important sources of N input. For this reason, it is important to understand factors that influence N fixation and release in soil crusts. Given adequate soil nutrients and alkaline pH, the major factors controlling N-fixation rates are temperature, light, and moisture. As crust organisms are only active when wet, moisture is the most limiting factor. When sufficiently moist, access to intracellular carbon stores then controls fixation rates. Because of this reliance on carbon stores, N-fixation rates are highly dependent on conditions experienced prior to experimental measurements. With sufficient hydration and access to carbon stores, temperature controls fixation rates.

Other factors also influence N-fixation rates. High soil salinity, high soil N, and low pH can depress fixation rates. Phosphorus additions can stimulate fixation rates. Micronutrients have been shown to both increase and decrease fixation rates. Association with other species has also been shown to influence N-fixation rates. Bacterial symbionts may increase rates. Associations with mosses, lichens, and higher plants can both increase or decrease rates, depending on conditions.

Much of the N fixed by crustal species is released soon after fixation. Released N has been shown to be utilized by surrounding organisms, including vascular plants, fungi, actinomycetes, and bacteria. Little is known about denitrification or volatilization losses from biological soil crusts.

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