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MICROBIAL STRESS-RESPONSE PHYSIOLOGY AND ITS IMPLICATIONS FOR ECOSYSTEM FUNCTION

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Abstract. Microorganisms have a variety of evolutionary adaptations and physiological acclimation mechanisms that allow them to survive and remain active in the face of environmental stress. Physiological responses to stress have costs at the organismal level that can result in altered ecosystem-level C, energy, and nutrient flows. These large-scale impacts result from direct effects on active microbes' physiology and by controlling the composition of the active microbial community. We first consider some general aspects of how microbes experience environmental stresses and how they respond to them. We then discuss the impacts of two important ecosystem-level stressors, drought and freezing, on microbial physiology and community composition. Even when microbial community response to stress is limited, the physiological costs imposed on soil microbes are large enough that they may cause large shifts in the allocation and fate of C and N. For example, for microbes to synthesize the osmolytes they need to survive a single drought episode they may consume up to 5% of total annual net primary production in grassland ecosystems, while acclimating to freezing conditions switches Arctic tundra soils from immobilizing N during the growing season to mineralizing it during the winter. We suggest that more effectively integrating microbial ecology into ecosystem ecology will require a more complete integration of microbial physiological ecology, population biology, and process ecology.

Key words: Alaska; arctic tussock tundra; microbial communities; microbial physiology; soil processes; stress.

Introduction

A changing environment creates conditions that can be stressful for microorganisms, and they are neither immortal, nor impervious to stress. Microbes must have physiological acclimation mechanisms to survive and remain active in the face of stress or they will die. However, those adaptation and acclimation strategies create physiological costs at the organism level and can alter the composition of the active microbial community (Fig. 1), creating shifts in ecosystem-level C, energy, and nutrient flows.

Only a few studies have tried to develop the complex flow of influence from environmental conditions, through microbial physiological responses, community composition changes, and on to the ultimate ecosystem-scale dynamics (e.g., Zak et al. 2003, Balser and Firestone 2005). This stands in contrast to plant ecology, where understanding specific organismal responses to environmental stress has been central to the field and to integrating population and ecosystem ecology. For example, Grime's (1977) characterization of plants as colonizers, competitors, or stress tolerators defines life strategies that encompass the physiological traits that control species' influences on ecosystem processes: litter chemistry and nutrient content, leaf longevity, secondary chemistry, etc. (Chapin et al. 2002).

We argue that in developing a stronger connection between microbial and ecosystem ecology, an enhanced understanding of microbial physiological responses to stress may be as important as it has been in understanding plant ecology. How do stress tolerance physiologies vary among microbial groups? How do those ramify into ecosystem-level consequences in response to stress? We first consider some theoretical aspects of the nature of stress at the microbial scale and general patterns of microbial responses to stress. We then consider how two common and important ecosystem-level stressors, drought and freezing, alter microbial physiology and community composition, and through these, ecosystem C and N flows.

At the ecosystem scale, "stress" is usually considered to be a chronic challenge (e.g., drought, toxins, and so forth) that imposes physiological costs, while "disturbance" is usually seen as a pulse event that primarily involves physical disruption and direct mortality (fire, windstorms, harvest, and so forth). At the microbial scale, this distinction is less clear: microbes are likely to experience ecosystem-scale physical disturbances pri-

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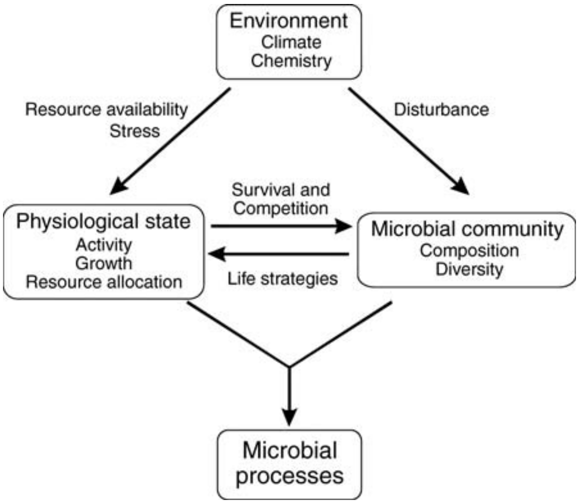

FIG. 1. Links among environmental drivers, microbial physiology, community composition, and ecosystem processes.

marily through altered micro-climate and resources, and thus as physiological challenges. The exception might be events that disrupt soil structure (e.g., tillage or whole-tree harvesting), which are likely to affect primarily fungi, whose mycelia can be broken up. On the other hand, microbes commonly experience pulse changes in the state of water, such as drying/rewetting and freeze/thaw. These pulses may be more challenging than the chronic stress they are associated with (e.g., drought, freezing [Kieft et al. 1987]), but their primary effects are physiological, not physical. In this paper, we consider "stress" to be something that creates physiological challenges that threaten microbial function or survival.

Microbes must acclimate to immediate stress by altering their allocation of resources from growth to survival pathways (Fig. 2); a stress too extreme will force them into dormancy (Farrar and Reboli 1999, Suzina et al. 2004) or kill them. Death and dormancy both remove microbial function from the soil, but, whereas dormant organisms regain activity when conditions improve; dead ones do not. If microbial death is extensive, dispersal and regrowth become important in regulating ecosystem function, and serious time lags and disconnections between processes may occur (Clein and Schimel 1994). For example, methane consumption may take years to recover after lethal events (Mosier et al. 1997). In dying, microbes also release carbon and nutrients that may be leached from the soil (Miller et al. 2005), taken up by plants (inorganic phosphate, amino acids, and so forth [Schimel and Bennett 2004]), or used by other microbes. These microbes may use the material to support growth and survival (cryptic growth [Chapman and Gray 1986]), to enable attack on recalcitrant soil organic matter (priming [Fontaine et al. 2004]), or to fuel processes such as denitrification (Sharma et al. 2006).

Even modest, "tolerable" stresses may have substantial influences on ecosystem functioning. Such stresses

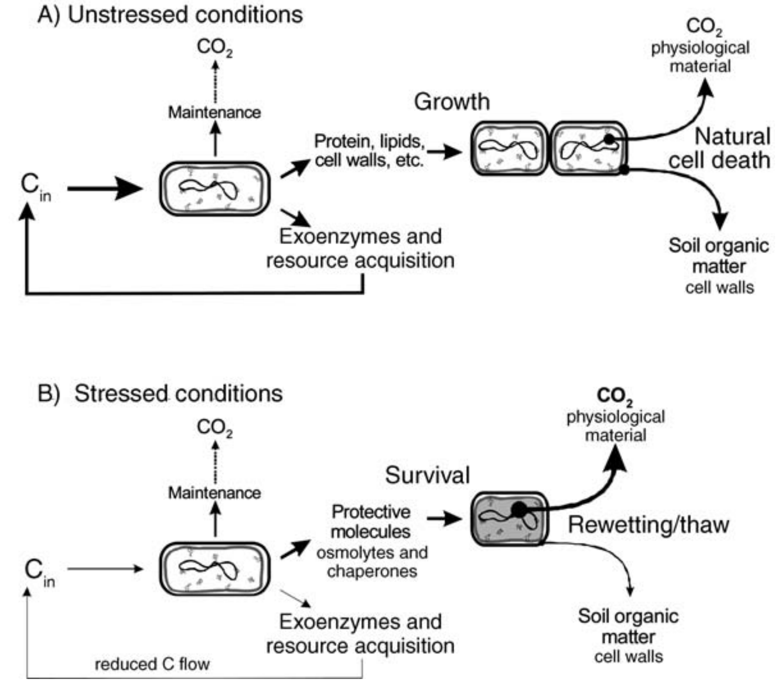

FIG. 2. Resource allocation patterns in microorganisms under (A) unstressed and (B) stressed conditions. Under stress there is a reallocation of resources from growth pathways to producing protective molecules. Ultimately, this makes substantial amounts of C and N vulnerable to loss.

impose C and N costs on microbes that they must meet to survive and remain active. The nature of those costs depends on the life strategy of microbes: are they inherently resistant to the stress or must they actively acclimatize to it?

Inherent resistance involves evolutionary selection of a growth form and life history strategy that allows a microbe to tolerate stress without having to induce specific mechanisms at the time of the stress. Developing such "toughness" invariably involves physiological trade-offs that affect microbial function, however. These trade-offs are likely analogous to those faced by plants that have evolved in low nutrient or physically stressful environments. Such plants typically allocate resources to high root biomass, nutrient poor foliage, and physical and chemical defenses and as a result have low maximum growth rates and a limited capacity to respond to "enhanced" conditions (Chapin et al. 1986). In the realm of microorganisms, for example, gram positive bacteria (with a strong, thick, interlinked peptidoglycan cell wall) are thought to be more inherently resistant to drying/rewetting events than gram negative bacteria (with a single-layer cell wall and an outer membrane). Producing such thick walls is expensive in terms of C, N, and energy inputs.

Acclimation, on the other hand, allows a microbe to induce specific tolerance mechanisms in response to stress. The ongoing costs for maintaining the genes necessary to induce resistance mechanisms are minimal, but those mechanisms may be energetically expensive to induce. Acclimation requires a microbe to redirect energy and nutrients from resource acquisition and growth into survival (Fig. 2), such as synthesizing chaperones to stabilize proteins (Yerbury et al. 2005) and osmolytes to reduce water potential and maintain hydration (Csonka 1989). The costs associated with these mechanisms may be large, and relying on acclimation carries the risks that either the needed resources will not be available, or that the stress will be too rapid for an organism to acclimate. Microbes can adapt to stress over extended periods (years to decades) by altering their genetic capacities (Walker et al. 2006), becoming either better able to acclimate to stress or enhancing their function after doing so.

MOISTURE STRESS

Drought

Drought is perhaps the most common environmental stress that soil microorganisms experience. One third of the earth's cover is arid, semi-arid, or seasonally arid (Mediterranean, dry tropical forest, and so on [Gurevitch et al. 2002]) and other ecosystems experience regular drought and episodic dry/rewetting cycles as well. As soils dry, substrate diffusion becomes limited and microbes may experience resource limitation that can slow biogeochemical process rates (Stark and Firestone 1995). However, decreasing water potentials impose direct physiological stress that forces microbes to shift resource allocation (Fig. 2), potentially altering the nature of C and N flows, rather than just slowing them down.

Microbes are small, in intimate contact with soil water, and have semipermeable membranes. Thus, cellular water potential rapidly equilibrates with that of the surrounding water. As soils dry and water potentials decline, cells must accumulate solutes to reduce their internal water potential to avoid dehydrating and dying (Harris 1981). As their primary osmolytes, microorganisms use simple organics with a good balance of high solubility and limited direct physiological effects (Csonka 1989). Bacteria typically use amino compounds such as proline, glutamine, and glycine betaine (Csonka 1989). Fungi, on the other hand, use polyols such as glycerol, erythritol, and mannitol (Witteveen and Visser 1995).

Accumulating solutes is energetically expensive. Bacteria can accumulate amino acids to concentrations of roughly 0.5 mol/L and these may account for between 7% and 20% of total bacterial C (Koujima et al. 1978, Killham and Firestone 1984*b*) and between 11% and 30% of bacterial N. In fungi, identifiable polyols can account for over 10% of cell mass (Tibbett et al. 2002); because polyols do not contain N, N costs are low. In addition, a range of other molecules may be produced under moisture stress; under extreme conditions, total cytoplasmic constituents can increase to as much as 30–40% of total C for both bacteria and fungi and almost 60% and 20% N, respectively, compared to only 3–6% of cellular C and N under unstressed conditions (Schimel et al. 1989). The values for C do not include the C used to provide the energy needed to produce the osmolytes. Under optimal conditions, microorganisms can only assimilate between 10 and 50% of the carbon they take up, depending on the specific chemicals (Sugai and Schimel 1993); the total C cost is easily, therefore, two to three fold greater than the simple accumulation of C compounds. In fact, osmotic stress can reduce growth yields (the amount of biomass produced per gram C metabolized) by roughly 90% (Killham and Firestone 1984*a*).

When cellular C and N demands for osmolytes are extrapolated to an ecosystem scale, the amounts are large. For example, consider a grassland soil with a microbial biomass of 800 μg C/g soil to 10 cm (global average for grassland and forest soils [Wardle 1998]), bacteria are 75% of the microbial biomass (Allison et al. 2005), osmolytes are 10% of microbial C, bacteria use entirely amino acids, while fungi use entirely polyols. Using these assumptions, osmolyte C would be on the order of 10 g C/m^2. If we assume a substrate use efficiency of 50% to synthesize the osmolytes that translates to a C cost of 20 g C/m^2, excluding other compounds that may accumulate. That value compares to total NPP values for grasslands that are regularly in the range of 300–600 g C·m^{-2}·yr^{-1} (Gurrevitch et al. 2002). Thus, at a conservative estimate, the total C cost of producing osmolytes in a single drought period can easily consume 3–6% or more of total ecosystem annual net primary production (NPP) in a grassland ecosystem. The total N contained in osmolytes would be 1.75 g N/m^2 or more, a value that would represent 10–40% of annual net N mineralization (Burke et al. 1997).

The above calculations suggest that the total resource demand for synthesizing osmotic protectants is large. One would expect that at the end of a moist period, microorganisms would have used most of the available resources in synthesizing biomass. Bacteria only store substantial amounts of C when N is limiting and they do not appear to store N (Banham and Whatley 1991, Lee et al. 1999). This raises several questions: how do microbes acquire the C and N to produce the necessary osmotic agents? What do microbes do if they do not have the available resources? Do microbes die from stress really because they don't have the resources needed to acclimate?

Rewetting

Having accumulated osmolytes, microbes are faced with the challenge of disposing of them when the soil rewets. Soil rewetting is rapid and if a microbe does not dispose of its osmolytes, water will flow into the cell, potentially causing it to rupture unless it has strong cell walls (Kieft et al. 1987). To prevent this, microbes must dispose of osmolytes rapidly on rewetting, either by respiring, polymerizing, or transporting them across the cell membrane (Wood et al. 2001). For example, Kieft et al. (1987) showed that as much as 50% of the microbial biomass could be released on upon rewetting, although probably little of this is from cell death (Halverson et al. 2000).

One ecosystem-level consequence of disposing of osmolytes is a pulse of CO$_2$, DOC, and nutrients released on rewetting (Birch 1958, Clein and Schimel 1994, Scheu and Parkinson 1994, Steenwerth et al. 2005). That pulse is made up largely of microbial cellular material (Fierer and Schimel 2003), and its magnitude varies between 7% and 40% of the size of the microbial biomass (with a mode in the 10–20% range [Van Gestel et al. 1993, Pulleman and Tietema 1999, Fierer and Schimel 2003]).

The specific cost in terms of lost osmolyte C from a single drying/rewetting event is modest, but when repeated multiple times, as is common in many ecosystems, this pulsing can pump C out of an ecosystem. Multiple drying/rewetting events increased soil respiration by 60% over soils that were kept at a constant optimum water content in a California chaparral soil (Miller et al. 2005). In a modeling study on a Belgian deciduous forest, pulse responses alone accounted for between 10% and 14% of annual CO$_2$ flux (Yuste et al. 2005), a result consistent with other modeling studies (Li et al. 2006). Were the C metabolized in rewetting pulses used for cell replication, rather than stress tolerance, C would go into cell polymers (Fig. 2), including cell walls, that are much more likely to be stabilized into recalcitrant soil C forms (Balser 2005).

Long term effects via community change

It is likely that in the short-term, direct effects on physiology are the dominant influences on soil processes, but changes in community composition may have strong long-term effects. For example, in decomposing birch leaves, a single drying–rewetting reduced respiration by 25% over the month following the event, suggesting a reduction in the capacity of the microbial community, possibly due to a loss of some decomposers (Clein and Schimel 1994) and extended drought produced a microbial population with lower respiration rates per unit biomass (Schimel et al. 1999). Apparently, the organisms that can survive drought have lower metabolic capacity, analogous to plants adapted to stressful environments (Chapin et al. 1986). Fierer et al. (2003) found changes in microbial processes six weeks after the end of a series of stress cycles, and these changes were associated with changes in the composition of the microbial community. Pesaro et al. (2004) found that while respiration rates recover quickly following drought, biomass and certain specific groups remain depressed for at least one month.

Shifts in community composition result because the costs associated with tolerating moisture and rewetting stresses fall differently on different organisms, depending on their inherent resistance and acclimation abilities. Harris (1981) distinguishes four microbial "functional" groups related to water stress: (1) no osmolytes (no acclimation), (2) inducible osmolytes only (pure acclimation), (3) constitutive osmolytes only (purely inherent resistance), and (4) both inducible and constitutive osmolytes production (mix of inherent resistance and acclimation). Most gram-negative bacteria appear to fall into category 2 and rely on acclimation strategies. Gram-positive bacteria have strong cell walls and so are generally inherently resistant, falling into groups 3 and 4. Fungi, too, would be more likely to be found in group 4. Group 1 appears to be rare in soil organisms.

The general morphology and life history strategies of microbial groups suggests that soil drought and the immediate rewetting shock should select against gram negative bacteria and for gram positive bacteria and fungi. This community sorting based on drought-tolerance strategies could be important because different groups of organisms carry different biogeochemical capabilities. Many organisms responsible for litter and soil organic matter breakdown are found in the drought-tolerant gram positives and fungi and so overall CO$_2$ flux might be only modestly sensitive to climate-induced community changes (Schimel 1995). However, many of the organisms carrying out "specialized" or "narrow" (sensu Schimel 1995) functions in soil, such as nitrifiers, methane oxidizers, and sulfur oxidizers are gram negatives and so may be more sensitive. Additionally,

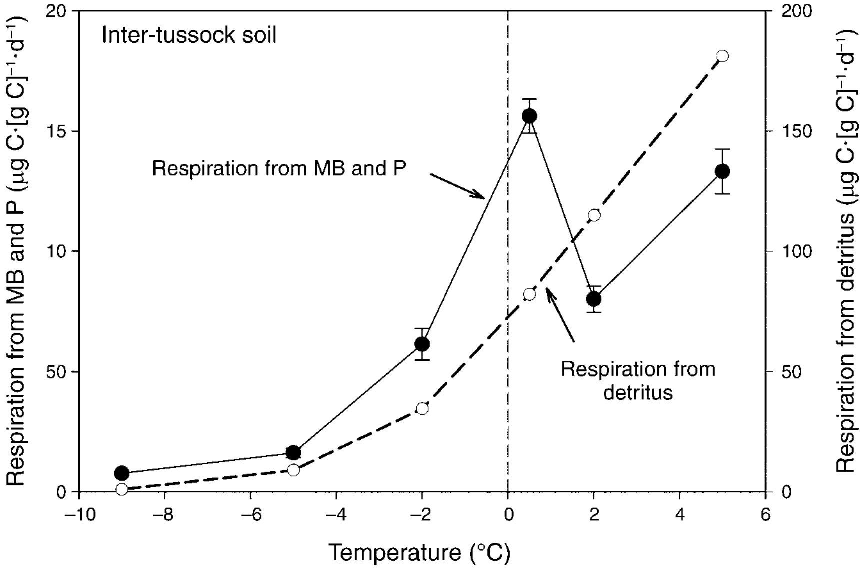

FIG. 3. Respiration from microbial biomass and products (MB and P) and from detritus in inter-tussock soil as temperatures drop from +5°C to −9°C. The figure is reproduced from Schimel and Mikan (2005).

while there are gram positive denitrifiers, there appear to be more gram negatives with the ability to denitrify (Shapleigh 2000).

Our hypothesis relating community change to stress-response physiology appears to be borne out to some degree. Fungi tend to be drought tolerant (Harris 1981) and drought tends to shift the bacterial community toward gram positives (Nazih et al. 2001, Uhlirova et al. 2005), although gram negatives that survive sometimes thrive in the period immediately following rewetting (Steenwerth et al. 2005). Additionally, microbial communities that experience regular episodic stresses appear more tolerant of those stresses than communities that do not regularly experience such stresses, suggesting selection for organisms with effective tolerance mechanisms (Van Gestel et al. 1993, Fierer et al. 2003, Steenwerth et al. 2005)

Trying to evaluate microbial community shifts in response to drought stresses simply on the basis of fungi, gram-positive bacteria, and gram-negative bacteria is clearly a very preliminary characterization, and full of exceptions (Scheu and Parkinson 1994), but provides a starting point for developing an integrated understanding of how drought stresses affect overall ecosystem processes through the interacting effects on microbial resource allocation and community composition (Fig. 1). We believe that there are analogues between a developing microbial community framework and ideas common in plant ecology. Van Gestel et al. (1993), for example, argues that the soil microbial community is comprised of drought-tolerant, but less active microbes, and a group of *r*-selected rapidly growing but drought sensitive microbes, with traits closely analogous to the stress tolerators and colonizers of Grime's (1977) plant classification system (Fierer et al. 2007).

FREEZING AND FREEZE–THAW

Freezing

Cold, particularly freezing, temperatures, are another common stress that requires microbial adaptations and acclimations (Walker et al. 2006). At low temperature, lipid membranes can solidify (Methe et al. 2005) and ice crystals can rupture cell membranes (Rivkina et al. 2000), both potentially fatal. Additionally, as bulk soil water freezes and only thin films of water remain on particle surfaces (Clein and Schimel 1995) substrate and O_2 diffusion decrease, inducing substrate limitation and anaerobiosis (Clein and Schimel 1995).

For microbes to survive freezing temperatures and remain active, there are a number of necessary physiological acclimations—they must shift biochemical pathways (Methe et al. 2005), alter membrane lipids to maintain membrane fluidity (Methe et al. 2005), synthesize protective molecules that include proteins and sugars (Mihoub et al. 2003, Kandror et al. 2004), synthesize antifreeze proteins (Bae et al. 2004), and possibly produce compatible solutes to control water potential (Ko et al. 1994; but see Mindock et al. 2001).

The ecosystem-level cost of inducing freeze-tolerance appears to be substantial. In Arctic tundra, as soil temperatures drop to near 0°C and microbes must induce cold-acclimation mechanisms, they increase their metabolism of material from the "microbial biomass and products (MB and P)" pool—the small, rapidly turning over, N-rich pool that is comprised of material from live and dead cells (Fig. 3; Schimel and Mikan 2005). This metabolic shift is large enough to change overall patterns of ecosystem C and N cycling from net N immobilization during the growing season to net mineralization during the winter, when all the measurable annual net mineralization occurs in Alaskan tussock tundra (Giblin et al. 1991, Schimel et al. 2004). An important component of this physiological shift is that it occurs above 0°C; thus, rather than waiting for the freezing stress to occur, microbes appear to pre-acclimate to the stress, analogous to frost hardening in plants (Lennartsson and Ogren 2002).

Freeze–thaw cycles

Few studies have isolated the effects of freezing temperatures alone because it is generally necessary to thaw the soil to analyze it. Thus, most of our available knowledge is about the integrated effect of freeze–thaw cycles. As with rewetting a dry soil, thawing a frozen soil requires microbes to rapidly reverse their physiological acclimations to freezing to prevent cell rupture and other effects. These responses invariably release a flush of C and N, though some of this material may be from cells killed by freezing and only made available to other microbes on thaw. The pulse of respiration on thaw can be equivalent to 5–15% of the total microbial biomass (Skogland et al. 1988, Herrmann and Witter 2002), a proportion similar to that released on rewetting (Skogland et al. 1988). As with rewetting, the actual respiratory burst is likely an underestimate ($\sim$50% or more) of the extent of the microbial C mobilized, since not all the released C is respired. In Arctic tussock tundra with annual aboveground net primary production of 35 g $\mathrm{C \cdot m^{-2} \cdot yr^{-1}}$ (Chapin and Shaver 1985), 3800 g C in the O horizon (Mack et al. 2004), an average microbial biomass of 25 mg C/g soil C (Cheng et al. 1998) and a thaw loss of 10% of biomass C, this would translate into a C loss of 9.5 g C/$\mathrm{m^2}$ from a single thaw pulse, or roughly 25% of annual aboveground net primary production.

The release of N associated with freeze/thaw can also be quite large. Estimates of N mineralization associated with a single freeze–thaw cycle can be as large as 60 μg N/g organic matter (Schimel and Clein 1996) in Arctic tussock tundra soil. That translates to roughly 0.5 g N/$\mathrm{m^2}$, a value five times the annual net mineralization rate of 0.1 g N/$\mathrm{m^2}$ (Giblin et al. 1991), although the mineralized N is rapidly reimmobilized in situ (Schimel et al. 2004).

Long-term effects through community change

Repeated freeze–thaw cycling can reduce the microbial biomass by as much as one-third (Larsen et al. 2002, Pesaro et al. 2003), and basal respiration by up to 30% (Schimel and Clein 1996). Damage is usually greater when microbes are active and growing (Schimel and Clein 1996). When soils experience multiple freeze–thaw cycles, most of the vulnerable biomass appears to be lost in the first cycle and microbes that survive the first cycle are resistant to additional events (Larsen et al. 2002, Walker et al. 2006). Such results suggest that different groups of microbes have different growth strategies when it comes to responding to freezing. In contrast to drought, one might hypothesize that bacteria are more tolerant than fungi because the mycelial growth form should be sensitive to the physical disruption associated with freezing. Multiple freeze/thaw cycles appear to shift communities toward increased bacterial dominance (Nieminen and Setala 2001, Larsen et al. 2002) as bacteria survive while eukaryote and archaeal populations are reduced (Pesaro et al. 2003). In contrast to these studies, the gram negative *Pseudomonas*/*Alcaligenes* group appears to be sensitive to freeze/thaw (Skogland et al. 1988), although *Pseudomonas paucimobilis* was tolerant of a first freeze/thaw cycle but only sensitive to a second (Morley et al. 1983), suggesting it may have consumed the resources needed for acclimation in surviving the first freeze cycle. Although fungi are vulnerable to freezing, they sometimes increase under prolonged frozen conditions (Schadt et al. 2003, Sjursen et al. 2005). Since fungi are generally tolerant of low water potentials and are able to bridge gaps to tap spatially separated resources, this wouldn't be surprising.

There is little information about how low temperatures affect microbial community structure in nature. The one relatively complete picture of microbial winter dynamics is from the Colorado alpine where the microbial community cycles between a summertime, bacteria-dominated community and a winter, fungi-dominated community (Schadt et al. 2003); this community shift produces a flush of nutrients that support springtime plant growth (Schmidt and Lipson 2004). However, in the alpine, with thick snow cover, soil temperatures are never extremely low and the shifts in microbial communities are thought to be driven more by changes in available substrates—root exudates and other fresh, simple compounds in the summer, and more complex and recalcitrant litter-derived materials in the winter (Schmidt and Lipson 2004, Schmidt et al. 2007). Although this work has been cited as characterizing "tundra" (Neufeld and Mohn 2005), arctic and alpine tundra differ in many ways and it is quite possible that arctic communities have different patterns of microbial community dynamics.

Whereas surviving drought appears to be physiologically expensive, microbes appear generally able to do so. Freezing appears to be a greater challenge, possibly involving more actual mortality, although many of the direct physiological costs of surviving dry and freezing conditions appear similar in magnitude. The ecophysiological costs of both, and their effects on functional community dynamics, need to be better constrained at the whole-ecosystem scale.

CONCLUSIONS

Although we have only discussed only two specific stresses, drought and freezing, many stresses induce common physiological responses in microbes (Duguay and Silhavy 2004). Thus, these other stresses (e.g., toxins, heat, anaerobiosis, and others) should alter microbial resource allocation in ways similar to those identified in Fig. 2: a shift from growth to acclimation and survival-related metabolism, with similar consequences for ecosystem-level C and N flows. Additionally, there is interaction among stressors; actively growing organisms are more vulnerable to stress, so one stress that reduces growth rates can thereby induce tolerance to other stresses (Colucci and Inniss 1996, Robinson 2001). The influences of all types of stress operate at both physiological and community composition levels and these interact to produce the overall linkage between environmental conditions and biogeochemical processes (Fig. 1). While the physiological effects likely regulate short-term responses of soil communities and processes, shifts in community composition are likely to regulate them over longer periods. The importance of stress in structuring the composition and function of soil microbial communities suggests that our current conception of microbial "functional groups" based largely on process-based groups such as nitrifiers or denitrifiers, requires an additional dimension of environmental response, such as drought tolerators, or cold acclimators, to fully account for microbial process responses to environmental change. This matrix of function and environmental response creates the niche space that presumably drives the patterns of microbial diversity found in soil.

Developing the full flow of the linkage between environment and processes via physiology and community composition has been a major theme in plant ecology over the last decade or more (Chapin et al. 2002). Achieving the goal of effectively integrating microbial ecology into ecosystem ecology will require a similar focus within microbial research, integrating microbial physiological ecology, population biology, and process ecology, and evaluating how they are manifested at the ecosystem scale. While developing a synthesis continues to be a major challenge, we believe that it is not only achievable, but critical in advancing our understanding of ecosystem ecology.

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