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Unearthing ectomycorrhizal dynamics

We are in the midst of a revolution in field research of microbial ecology (Horton & Bruns, 2001). Historically, soil biologists have been hampered by difficulties inherent with working on microscopic organisms below ground. However, recent advances in molecular approaches have greatly facilitated our ability to survey microbial communities *in situ*. These techniques have become relatively inexpensive, and the capacity to process large numbers of samples has increased. Together, these two trends allow investigators to conduct field studies of ever-increasing scope. In this issue, Izzo *et al.* (pp. 619–629) demonstrate how these new capabilities can be used to glean foundational knowledge regarding microbial community structure – their work addresses the question as to what extent ectomycorrhizal communities change over time in an otherwise stable ecosystem. The research is groundbreaking in terms of its comprehensive and simultaneous measurement of spatial and temporal variability in ectomycorrhizal community composition.

Izzo *et al.* sampled at intervals ranging from 5 cm to 200 m at two depths over 3 yr. This combined approach was useful, because the authors found that the ectomycorrhizal community turns over frequently at smaller scales, but much less so than at larger scales. These results indicate that the pool of available ectomycorrhizal species within an ecosystem may remain more or less constant, whereas the exact location of individual species may shift over time. Had the authors focused on the contribution of either time or space, but not both, they would not have detected this pattern. The project of Izzo *et al.*, which was initiated in 1999, required the genetic analysis of a total of 1300 ectomycorrhizal root tips in all. Current macro- and microarray approaches now allow us to characterize thousands of samples at a time in runs lasting one month or less (e.g. Valinsky *et al.*, 2002). Molecular techniques are now a tractable tool for in-depth study of mycorrhizal communities.

The importance of community composition

Assessments of mycorrhizal communities are important because a growing body of research suggests that mycorrhizal species vary in their influence on a number of ecological processes. For instance, ectomycorrhizal species differ in the types and amounts of extracellular enzymes they exude into the soil to metabolize organic material (Read & Perez-Moreno, 2003). In turn, the growth rate and nutrient status of plants associated with different species vary according to the availability of particular soil organic compounds (Abuzinadah & Read, 1989). Correspondingly, certain ectomycorrhizal species can have markedly distinct effects on total soil carbon pools. An example of this scenario was documented by Chapela *et al.* (2001), in which an introduction of *Suillus luteus* in Ecuador resulted in a depletion of up to 30% of soil carbon stocks. Species effects can extend even to the atmosphere – ectomycorrhizal species differ in rates at which they emit methyl halides (Redeker *et al.*, 2004), which contribute to ozone destruction. This variation among species in ecological functions suggests that ectomycorrhizal community composition can influence each of the main components of ecosystems: plants, soils, and the atmosphere. The questions addressed by Izzo *et al.* therefore have a bearing on fields as broad-ranging as plant ecology, ecosystem science and atmospheric chemistry, as well as community ecology.

Rates of change

Given the potential for ectomycorrhizal community composition to influence the environment, an equally important issue involves how quickly shifts in composition can be expected to occur in response to natural environmental variation. Izzo *et al.* found that interannual variation in climate and other factors had little impact on the composition of the ectomycorrhizal community within the forest as a whole, even over 3 yr. By contrast, short-term changes pointed to the growth or death of particular individuals, rather than the loss or immigration of species. This distinction is important because it implies that the short-term changes may be easily reversible, depending on future conditions. We can contrast these results with those documented in disturbed ecosystems, in which changes in ectomycorrhizal composition have been recorded within a few years after exposure to fire, elevated CO_2, or nitrogen additions (Grogan *et al.*, 2000; Treseder & Allen, 2000). Most of these studies were conducted over larger spatial scales, usually more than a few meters. Are these shifts in community composition due to alterations in an otherwise stable 'background' pool within the ecosystem, as might be implied by the Izzo *et al.* study? Or are they the result of easily reversible changes in population dynamics?

Controls over biodiversity

Izzo *et al.* identified 100 local ectomycorrhizal species in their study, which is typical of this relatively diverse fungal group. What mechanisms might maintain this level of biodiversity? Could plants cultivate diverse ectomycorrhizal communities that vary in nutrient acquisition or seasonality? Again, to address these issues, detailed studies of community composition are required. Izzo *et al.* suggested that seasonal drought and root turnover may have contributed to their observed shifts in community structure. Studies within microcosms have indicated that high microbial diversity can be maintained if microbial groups vary in resource requirements and competitive ability, but only if the environment is spatially

heterogeneous (Rainey *et al.*, 2000). In the case of ectomycorrhizal fungi, the growth and death of roots may constitute a form of spatial heterogeneity. Horner-Devine *et al.* (2004) recently described the first known taxa–area curve for bacteria, which appeared to result from environmental heterogeneity rather than geographic distance. Likewise, Izzo *et al.* reported that neighboring ectomycorrhizal communities were more similar than were more distant communities. However, this relationship was only apparent in samples collected from upper soil horizons, and not for those from the lower mineral layer. Ectomycorrhizal diversity was also lower in the deeper horizons. It is possible that the lower layers are less heterogeneous in terms of nutrient availability or root colonization. Alternatively, a more uniform dispersal of ectomycorrhizal species at those depths could have been resulted from the reduced interannual variability in ectomycorrhizal community composition noted by Izzo *et al.*

Covering new ground

Basic characterizations of the identities of microbial species within communities can provide valuable information regarding community dynamics and ecological interactions. A number of molecular approaches can be used in these assessments, including: sequencing of individual ectomycorrhizal root tips (Izzo *et al.*); macro- or microarray analyses on bulk soil DNA to identify the presence of previously known species (Schena *et al.*, 1995), or to sort individuals into taxonomic groups (Valinsky *et al.*, 2002); and the use of taxon-specific primers on bulk soil DNA, coupled with real-time quantitative polymerase chain reaction (PCR), to measure abundance of particular groups of interest (Heid *et al.*, 1996). The next important step is to link community composition to function – this connection will allow investigators to test mechanisms and to predict ecological consequences of community shifts. Although in the past it has been challenging to use molecular information to determine species identity and function simultaneously, two new techniques may prove to be extremely useful in this regard. Stable isotope probing of DNA can be used to trace isotopically labeled nutrients directly from soil substrates into microbial DNA (Radajewski *et al.*, 2003). The labeled DNA can then be isolated by using density-dependent ultracentrifugation. Another option is nucleotide analog probing, in which soil samples are incubated with bromodeoxyuridine (an analog for thymidine) (Borneman, 1999). Microbes that are actively constructing DNA during the incubation period will incorporate the analog in place of thymidine in the DNA sequence. Analog-labeled DNA can then be separated via immunocapture. In this way, investigators can identify microbes that increase or decrease activity under varying conditions. Altogether, these approaches provide a promising means of understanding controls over microbial community structure, function, rates of change and biodiversity.

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