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Commentary

On the role of varying species combinations in microcosm experiments: how to test ecological theories with soil food webs?

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Testing ecological theories in soil systems has recently been encouraged by Wardle and Giller (1996) and by Ohtonen *et al.* (1997). According to them, soil ecology could substantially contribute to the development of ecological theories by testing hypotheses dealing with, e.g., trophic-level dynamics or the relationship between species diversity and ecosystem function. To increase the success of this nascent trend in soil ecology, I propose that soil ecologists, aiming to test community-level theories, should consider the question recently raised by studies seeking the diversity-functioning relationship: i.e., how to select species for treatment levels that represent different food-web structures. Studies using non-randomly selected sets of species to represent diversity levels (e.g., Naeem *et al.* 1994) have been argued to be unable to distinguish the effect of species diversity *per se* from the effect of specific species combinations (André *et al.* 1994, Huston 1997). In response to this criticism, randomly selected dissimilar replicates within diversity levels have become common in biodiversity studies (*see* Naeem *et al.* 1996, Tilman *et al.* 1996, Wardle *et al.* 1997). However, the difficulty of separating the effect of a chosen species combination from the effect of a feature of a food web exists in all experimental studies using artificially established food webs. I suggest that one solution to this common problem is the usage of replicates of varying species combinations, as demonstrated in biodiversity studies.

Microcosm experiments are regularly used to reveal the nature of interactions between soil organisms, and the connection between mineralisation and biotic relationships (*see* reviews by Coleman *et al.* 1983, Verhoef & Brussaard 1990, Verhoef 1996). Since microcosm experiments are of great value in soil ecology and using them for testing ecological theories is generally encouraged (Kareiva 1989, Drake *et al.* 1996, Moore *et al.* 1996), they will likely form an essential part of the new trend of soil ecology contributing to ecological theories. Food webs created in microcosm studies are usually simple in their structure in order to keep the mechanisms understandable, and therefore the characters of the few chosen organisms may have a large impact on the obtained results (*see* Faber & Verhoef 1991, Mikola & Setälä 1998a). Consider a microcosm experiment testing a general hypothesis “microbial grazers limit microbial biomass in soil food webs”. The normal practise to approach the hypothesis is to create a microbial community using a few microbial species and to add a few microbivores to half of the microbial communities. Both the pure microbial community and the microbial community with grazers are replicated, and based on the obtained results it is inferred whether microbivores limit microbial biomass in soil food webs. However, this kind of experimental set-up is powerless to tell how big a share of results reflects the characteristics of the chosen species combination.

Simple microcosm experiments, including only a couple of species, are without doubt appropriate and efficient in clarifying the mechanisms occurring in soil food webs, such as the influence of soil pores (Elliott *et al.* 1980) or fungal growth mode (Hedlund *et al.* 1991) on the interaction between microbes and microbivores. However, when testing general community-level hypotheses, instead of studying specific mechanisms, it could be advantageous to use varying species assemblages within treatment levels to ensure as general results as possible. The most appropriate way to test community-level hypotheses is to manipulate natural food webs in the field, but since this is laborious and sometimes impossible with soil food webs, microcosm experiments are necessary. However, as microcosm food webs with a strictly controlled structure are doomed to contain only a subset of species living in the field, varying replicates are needed to ensure the generality of results. I suggest that by modifying the set-up of microcosm experiments — equal vs. varying replicates — microcosms can be effectively applied in soil ecology both to study mechanisms and to test general ecological theories (Table 1). Furthermore, experiments with varying replicates could provide valuable information of the dominance of species characteristics in simple communities, and consequently of the reliability of general patterns observed in simple microcosm communities.

Table 1. Replicates of two treatments in microcosm experiments designed (a) to study a specific mechanism affecting the interaction between a fungus and a fungivore, and (b) to test a general hypothesis "fungivores control the biomass of fungi in soil".

Treatment 1	Treatment 2
(a) Equal replicates	
Fungus a	Fungus a + fungivore c
Fungus a	Fungus a + fungivore c
Fungus a	Fungus a + fungivore c
Fungus a	Fungus a + fungivore c
Fungus a	Fungus a + fungivore c
Fungus a	Fungus a + fungivore c
(b) Varying replicates	
Fungus a	Fungus a + fungivore a
Fungus c	Fungus c + fungivore b
Fungus b	Fungus b + fungivore c
Fungus c	Fungus c + fungivore b
Fungus d	Fungus d + fungivore a
Fungus a	Fungus a + fungivore c

The hypothesis "microbial grazers limit microbial biomass in soil food webs" has, at least implicitly, been tested several times, and the response of microbial biomass to grazing has varied greatly. When being grazed, the biomass of bacteria has either been similar (Brussaard *et al.* 1995, Mikola & Setälä 1998b), lower (Woods *et al.* 1982, Allen-Morley & Coleman 1989) or higher (Abrams & Mitchell 1980, Ingham *et al.* 1985, Griffiths 1986) than in control systems without grazers. Similarly, the biomass of fungi has either decreased (Hanlon & Anderson 1979, Ingham *et al.* 1985, Allen-Morley & Coleman 1989) or increased (Bååth *et al.* 1981, Bengtsson & Rundgren 1983, Faber *et al.* 1992, Hedlund & Augustsson 1995, Mikola & Setälä 1998b) when grazers have been added. The variable results imply that the ability of grazers to limit microbial biomass is not straightforward in soil food webs. However, it is hard to interpret whether the strength of limitation depends on the species combinations used or whether it is, for instance, a consequence of different abiotic conditions in the experiments. The advantage of using varying species combinations as replicates is to increase the possibility that results of an experiment represent the average pattern looked for, and are not caused by hidden treatments (sensu Huston 1997) such as species combination. Replicates do not need to be selected entirely randomly to achieve the required effect, for replicates can as well include, for instance, all combinations that can be formed of available species. All that matters is that replicates are different from each other and thus delete the influence of a specific species combination.

The benefits of random replicates become also clear in the light of recent food-web studies which suggest that most interactions among populations are weak and only a few are strong. For instance, Paine (1992) showed that only a minority of algal grazers in an intertidal zone had a considerable negative effect on algal density. Similarly, Wardle *et al.* (1995) found that in a decomposer food web the abundance of predatory nematodes was closely connected to the density of only one of the several potential prey sources. If we select species for a microcosm experiment from a community with this kind of highly skewed distribution of interaction strengths, the results of the experiment probably become erroneous in either of two ways. Firstly, without *a priori* knowledge of interaction strengths we probably, by chance, select a few species with weak interactions. Secondly, if we have *a priori* knowledge of strong interactors, we probably choose them to get interesting results in the experiment (Lawton 1992). Contrary to these two procedures, varying species combinations as treatment replicates probably produce a few replicates with a strong interactor and several replicates with weak interactors only. The replicates of an experiment thus mirror the distribution of interaction strengths in natural communities and the treatment response tells more of the average influence which is looked for. Moreover, experiments with varying replicates can point to species whose interactions require further experimental studies with a set-up designed to study specific mechanisms.

Compared with experiments using equal replicates, experiments with varying replicates should produce a more accurate estimate of the pattern occurring in the field, but simultaneously the variance of the estimate increases. This fact has to be considered when determining the number of replicates, for more replicates are probably needed in experiments with varying replicates to achieve the same statistical power as in experiments with equal replicates.

Besides using dissimilar replicates to affirm the genuineness of observed patterns, results from separate experiments can be collected and considered afterwards. A good example of this approach is provided by Brett and Goldman (1997) who conducted a meta-analysis of independent mesocosm experiments to ascertain whether the biomass of phyto- and zooplankton in freshwater food webs is limited by resources or predation. In fact, reviews of the effects of microbivores on microbial biomass have also been published (e.g. Ingham *et al.* 1985), but statistical tests, assuring the existence of a general pattern, are lacking.

To conclude, when the effect of food-web structure is studied in microcosm experiments, it is always mixed with the effect of the chosen species combination. One way to delete the species-combination effect is to conduct a meta-analysis of multiple experiments dealing with different species combinations. The only way to erase the effect "within an experiment" is to use dissimilar replicates within treatment levels. Although varying replicates have rapidly become common in biodiversity experiments, they have not been used in experiments containing several trophic levels and studying, e.g., trophic-level dynamics. Soil ecologists, therefore, have an opportunity to extend this methodological improvement into new areas of experimental ecology, especially as the microcosm technique provides, with a moderate effort, possibilities to establish an experiment with several food webs of a slightly different structure.

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