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Biodiversity and plant mixtures in agriculture and ecology

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ABSTRACT

Biodiversity experiments where species richness is experimentally manipulated and the functioning (most often productivity) of the mixtures is considered as a response are carried out in both agriculture and ecology. They have some common features (and common problems), although the interpretation of results might differ in ecology and agriculture. A common problem is the use of various indices of "biodiversity effect" in statistical analyses. Their values are based on the same set of monoculture values, and consequently, they are not real "independent observations", as is assumed in the statistical tests. This fact can lead to an inflation of Type I error rate. The interpretation of results is more straightforward in agriculture, where species composition is determined in both experiments and real agriculture by the experimenter and farmer respectively. In real ecological communities, species composition results from a balance of various ecological forces, especially dispersal (species pool), environmental filtering (which species are able to survive in a given environment), and biotic filter (which species are able to survive in competition). As a result, randomly generated species composition of mixtures in biodiversity experiments is not always realistic.

Keywords: biodiversity experiments, competitive exclusion, complementarity effect, overyielding indices, sampling effect.

INTRODUCTION

The belief that species-rich communities function better (e.g. are more efficient in nutrient uptake, have higher productivity, are more stable) is very old and can be traced back to Darwin. For example, the belief that diversity begets stability was so strong in the fifties and sixties of the twentieth century, that the well-known Shannon index of diversity was introduced in ecology by MacArthur (1955) as an index of stability. The logic behind this was that the more pathways for energy flow, the less vulnerable will the system be to loss of any of the pathways, and consequently, its functioning will be more stable.

The subject was very popular in the sixties, then it was nearly forgotten in the eighties, but in the early nineties new interest in this topic emerged. This interest was initiated by an experimental approach, started with the well-known Ecotron experiment (Naeem et al. 1994), which manipulated species diversity and took ecosystem functioning as a response. This approach was not entirely new – similar experiments had been carried out both in agriculture and in ecology earlier (e.g. Vandermeer 1992, Austin et al. 1985), but were usually analysed and interpreted in a different way.

In the last decade, many biodiversity experiments have tried to demonstrate that species-rich communities are either more productive or more resistant to invasion, or are more stable. In biodiversity experiments, species richness of plant mixtures is manipulated (i.e. mixtures varying in richness are sown) and functioning (most often productivity, but also nutrient uptake, resistance to invasions) is taken as a response. Then, in the data analysis, the productivity or another measure of ecosystem function is regressed on the mixture diversity. A positive relationship is then interpreted as a "biodiversity effect". This seemingly straightforward approach has some limitations (Huston 1997). The aim of this paper is to discuss some of the limitations, and to compare the interpretation of the experiments in ecology and in agriculture.

EXPERIMENTAL DESIGN

The typical experimental design is based on a closed set of species. Each of them is then grown in a monoculture, and then in mixtures of increasing species richness, up to mixtures containing all the species. It is important that all the species are equally represented at all the species richness levels; otherwise it is not possible to distinguish the species effect from the diversity effect, see Huston's (1997) critique of the Ecotron experiment. Here I will assume that all the species in the mixture are introduced in the same amount. Changing proportions of species is also a very useful approach, bringing new insights, separating the effect of the number of species from the effect of evenness. Those might be investigated using the *simplex* design (Cornell 1990).

Even if we stick with equal proportions, some options remain to be decided. First, what does "equal amount" mean? When individuals are planted, then probably the same number of individuals of each species will be introduced. When species are sown, we have two possibilities – the same number of seeds, and the same total weight of seeds. The same number of seeds might favour plants with large seeds, and this might be particularly important when the plants are sown very densely. There might be a strong thinning at the very early stages of germination and seedling establishment, and the large seeds have a clear advantage. The same weight of the seeds seems to be a solution. However, not always. In favourable conditions (particularly not crowded, not very dense sowing), the proportion of seeds getting established might be relatively independent of the seed mass. If the same total weight of seeds is introduced, the number of individuals of species with small seeds will be larger than the number of individuals with large seeds. Various solutions have been suggested (e.g. to compensate with regard to the average seedling mass and seed germinability), usually lying somewhere between the two above-mentioned possibilities. The situation is even more complicated; some species might germinate slowly, and the slowly germinating species is then competitively suppressed (e.g. Lanta & Leps 2006 for *Lathyrus pratensis*, a species with relatively large seeds). The results for similarly designed experiments have differed considerably when species were introduced by sowing without any further measure taken (Lanta & Leps 2006) and when the initial number of established individuals was kept constant (Spackova & Leps 2001). However, keeping the initial number of individuals constant is feasible just in a pot or in very small-scale experiments.

Another decision must be made: between the additive and replacement series design. In additive design, the species is introduced at the same density in monoculture and in all the mixtures (and consequently, the more species, the higher the total amount of seeds). In replacement series design, the total amount introduced is kept constant in all units, and divided among the constituent species. In most experiments, the replacement series is adopted; sometimes, because of possible problems with replacement series (Connolly 1986), the total density is varied as another independent factor.

MECHANISMS OF BIODIVERISTY EFFECTS

Most studies have clearly demonstrated that productivity (and various efficiency characteristics connected with productivity) increases with species number. There are basically three mechanisms behind this pattern (Leps 2005).

1. **Sampling effect**

With an increasing number of species in the mixture, the chance that the mixture will contain a species able “to do the job” increases. Typically, if we have a productive environment and a set of (say) six species, of which only one is highly productive (e.g. *Chenopodium album*) and the others are fairly less productive (e.g. *Capsella bursa-pastoris* and similar species), then only mixtures containing *Chenopodium* will be highly productive, the others will be rather less productive. If sown in sufficiently high densities, *Chenopodium* will dominate in all the mixtures where it was sown, and those mixtures will have a high productivity, similar to that of a *Chenopodium* monoculture, the others will be rather less productive. In this case, one sixth of the monocultures, one half of the three-species mixtures, and all the six-species mixtures will contain *Chenopodium*. As a consequence, the average productivity will increase with species richness. In those cases, the maximum productivity for given species richness usually remains constant, whereas the minimum increases with species richness (Spackova & Leps 2001). The terms “chance effect”, or “selection effect” have a similar meaning as sampling effect. It can be shown that the sampling effect can cause only non-transgressive overyielding (i.e.

the mixture produces more than would be expected from monoculture yields), but not transgressive overyielding (i.e. the mixture produces more than the most productive of monocultures of constituent species). It has been questioned whether this is a real ecological mechanism or just an artefact of the sampling design (Huston 1997).

2. **Complementarity effect**

This effect is caused by the differential usage of resources by different species (their *niche differentiation*), leading to more efficient total use of the resources. As an example, the different rooting depths of the individual species can lead to better nutrient uptake by the community. Another possibility is temporal niche differentiation. For example, if two species, one with a peak early in the season, and the other with a peak late in the season will be grown together in an agricultural experiment with multiple harvests, then the cumulative yield of the mixture will very probably be higher than the cumulative yield of any of the two monocultures. Niche complementarity is a widely accepted ecological mechanism, and it is generally believed that it is one of underlying mechanisms of species coexistence. Complementarity can (but need not) lead to transgressive overyielding (i.e. the total productivity of the mixture is better than the productivity of all of its constituent species). Consequently, the absence of transgressive overyielding does not mean that complementarity does not play a role.

3. **Facilitation**

In the case of facilitation,the presence of one of the species improves the conditions for another one. A typical example is the presence of legumes improving the nitrogen conditions for grasses. This case can lead to transgressive overyielding, but it may also cause the productivity of a species in mixture to be higher than its productivity in monoculture.

In practice, facilitation is very difficult to separate from the complementarity effect without a detailed knowledge of the mechanisms, and both are thus often subsumed under the term “complementarity”.

DATA ANALYSIS

There are several possibilities of analysing data from biodiversity experiments. Here I will comment on some selected problems of data analysis.

First, the analyses depend on the type of data we have available, i.e. whether we have data on the productivity of individual species in mixtures or not. In many experiments, particularly those that work in a realistic setting (e.g. agricultural experiments on a realistic spatial scale in meadows), only the total productivity (biomass), and not that of the constituent species is available.

A frequent approach in such cases is the calculation of overyielding indices, where the actual productivity of the mixture is compared with that expected when the productivity of each species in the mixture exactly corresponds to its share in the mixture multiplied by its productivity in monoculture. There is a whole series of indices (see Garnier et al. 1997), but they all require the values for monoculture productivity of all the species to be available. If the biomass in mixtures had been separated into individual species, it is even possible to estimate the additive partitioning of the total biodiversity effect into a part caused by the Sampling effect and a part caused by the Complementarity effect (Loreau & Hector 2001). The values of indices are often taken as dependent variables in statistical analyses. Unfortunately, they are not independent observations (as is assumed in statistical tests), because each of them uses the same set of monoculture values. For example, if the productivity of *Chenopodium album* had been underestimated (just because of random error), then all the values of the biodiversity effect of mixtures with this species will have been overestimated (i.e. the values would have highly correlated errors). And yet, in statistical analyses the values are treated as independent. This might lead to Type I error inflation. How severe this danger is needs further investigation. Having well replicated values for monocultures is a way to reduce (but not completely eliminate) this danger.

However, the independence of observations is questionable even in cases when biomass is the dependent variable. There are two levels of “random” variability, one determined by the species composition of a mixture, and then the “true” random variability (local conditions of a replicate). Those two levels are usually not taken into account in statistical analyses.

INTERPRETATION OF RESULTS

The pioneering paper based on the Ecotron experiment (Naeem et al. 1994) was published under the title “Declining biodiversity can alter the performance of ecosystems“. In fact, the experiment demonstrated that the productivity of synthesised experimental microecosystems grown in Ecotron (a system of giant growth chambers) increases with the number of constituent species, but the results were interpreted in terms of current loss of species diversity in the Earth’s ecosystems and were seen as a good argument for nature conservation. This idea was explicitly formulated by Tilman (1999), commenting the results of the European Biodepth experiment (Hector et al. 1999). In his commentary, Tilman asked: “How much diversity might be needed to maintain high productivity within an ecosystem?” and concluded (on the basis of calculations using Species-Area relationships) that: “…a single hectare would have to contain about 60 to 105 plant species and 1 km^2 about 127 to 270 species for high productivity to exist. … This suggests that increasing diversity in managed grasslands and forests may be cost-effective”. This led to controversies, particularly because experience from most temperate seminatural grasslands shows that one of the main causes of their species loss is increased nutrient load and resulting increased productivity and competitive exclusion of subordinate species. In order to keep the diversity in seminatural grasslands high, we need to accommodate management which keeps productivity low (seminal example is the Rothamsted Park Grassland Experiment). Conservationists in Central Europe have for decades tried to persuade farmers to keep the productivity in localities of conservation interest low in order to preserve their diversity. Arguments that keeping diversity high could increase productivity might thus be counterproductive.

Endangered species in temperate seminatural grasslands exhibit mostly low productivity and low competitive ability (Leps 2004), and are confined to unproductive, oligotrophic meadows. Their loss in nature is caused either by increased nutrient load, leading to increased productivity and subsequent competitive exclusion, or by cessation of mowing, followed by the spread of strong competitors and, again, resulting competitive exclusion. Species that are lost are usually tiny, nonproductive species, like *Carex pulicaris, Pedicularis sylvatica,* etc. Low vegetative height was the best predictor of decline under productive conditions (Leps 1999). Experience shows that their loss can hardly affect the ecological “macrofunctions”, like productivity and nutrient cycling (at least not directly). However, many of them are food plants of specialised herbivores, and their loss causes further loss of biodiversity in a cascade effect (a typical example is the decline of the marsh gentian, *Gentiana pneumonanthe*, accompanied by the decline of its monophagous herbivore, *Maculinea alcon*).

Experiments with plant mixtures are carried out in both agricultural and ecological research. Whereas their experimental design is often similar, interpretations of the results might differ. The typical agricultural experiment deals with short-term crops. In this case, similarly as in real agriculture, the sown or planted species composition (including proportions of species) is determined by the researcher in an experiment, and by the farmer in the field. In long term experiments (permanent pastures or realistic ecological experiments), we should be aware that the initial species composition and proportions will change with time. Various restoration attempts show that it is easier to introduce the desired species to a site than to manage the system in a way enabling permanent coexistence of the introduced species. If we are interested in real ecological communities, then the species should be able to coexist for a long time. There is also a considerable difference in the range of species richness studied. In typical agricultural mixtures aiming at productivity, the species richness is usually low, e.g. two grasses and two legumes in sown pastures. Ecological experiments often have a much wider range of species richness values. If the experiments are aimed at mimicking current loss of species, we should work with highly diverse communities. For example, seminatural oligotrophic meadows that have been prone to species loss in recent decades often host up to 40 species per m^2 (Leps 1999). These communities are of high conservation value.

The functioning of ecological communities is affected by both the ecological characteristics of the constituent species (species traits) and by interactions among the species, as well as by limitations of the abiotic environment. Both the range of species trait values and the number and range of interspecific interactions increase with species richness. In nature, the species composition of a community and consequently also its species richness is determined by the local species pool, the abiotic environment, and biotic interactions in the community, which are also affected by the abiotic environment. (If we consider the plant community only, then its composition is affected not only by biotic interactions within the community, but also by biotic interactions with other trophic levels, both in the soil and aboveground.) Consequently, the three main limitations to species richness in nature are dispersal limitation (i.e. limitation by the available species pool), limitation by environment (mainly in harsh environments) and limitation by biotic interaction (often competition, mostly in productive environments). Biodiversity experiments account for changes in species variability caused by a changing species pool, but not by the two other factors. Consequently, the ecological properties of species-poor experimental mixtures are more indicative of natural communities which are poor in species because of a restricted species pool, rather than of those where low species richness is caused by an extremely harsh environment, or by competitive exclusion in a highly eutrophic environment.

The recent experimental approaches to the study of the effect of diversity on community functioning have brought new insights and have shown that on average, the productivity of communities increases with their species richness. However, there are also limitations that should be taken into account. The most important problems are: (1) The functioning of communities that are species-poor is determined by their limiting factor – communities in extremely nutrient-poor sands, on an eutrophized meadow and a distant island can all be similarly species-poor, but may behave in totally different manner. Experimental manipulation of species composition only models limitation by species pool. (2) In the real world, species lost are not a random selection of species present in the community. (3) Some species combinations (particularly some low diversity mixtures and monocultures) are extremely unlikely in nature. Similarly, in real agriculture, some species will not be used in monocultures (but can still be a useful part of mixtures). (4) In the meadows of Central Europe, the endangered and protected species are mostly those not able to increase the productivity of a meadow community. Biodiversity experiments are a useful tool in understanding the mechanisms of functioning of ecological communities. Interpretation of their results by nature conservationists should be very cautious, however, and, in my view, there are better conservation arguments than increased productivity in mixtures.

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