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Randomization tests for quantifying species importance to ecosystem function

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Summary

1. Quantifying the contribution of different species to ecosystem function is an important challenge. We introduce simple randomization tests (and software) for quantifying the average effect of species on ecosystem variables measured in multiple plots with and without the presence of a particular species. These randomization tests formalize the analysis of uncontrolled 'natural experiments' and quantify species effects in standardized deviation units.

2. We tested the method with data on ecosystem function in biological soil crust assemblages of lichens in semi-arid gypsum outcrops in central Spain. In sixty-three 50 cm × 50 cm sample plots, we measured the presence and percentage cover of 17 species of lichens and the levels of five important ecosystem variables (organic carbon, total nitrogen, urease activity, phosphatase activity and β-glucosidase activity). The randomization tests revealed 13 positive and six negative associations between species presence and ecosystem function.

3. We used data from an independent microcosm experiment on ecosystem function and species composition to validate these results. Microcosms that had higher levels of organic carbon and total nitrogen also had higher average species effect scores (measured from the survey data) for the species that were present in each experimental treatment.

4. As in all natural experiments, strong species interactions, effects of unmeasured abiotic variables on species occurrence and reciprocal effects of ecosystem variables on species occurrence can potentially confound estimates of species importance. Nevertheless, the method we propose provides a simple index and statistical test of species importance that can form the basis for additional hypothesis tests and experimental studies of species occurrence and ecosystem function.

Key-words: biological soil crust, lichen, natural experiment, null model, presence–absence matrix, randomization test

Introduction

A long-term research focus in community ecology has been to quantify the contribution of different species to ecosystem processes and function. Examples include studies of the effects of trees, shrubs and grasses on soil properties in a variety of ecosystems (see Binkley & Giardina 1998; Schlesinger & Pilmanis 1998; and Binkley & Menyailo 2005 for reviews), the role of herbivorous fish species in influencing coral reef development (Burkepile & Hay 2010) and the effect of particular functional groups, such as legumes, on biodiversity–productivity relationships in grasslands (Spehn *et al*. 2002), to name just a few.

Historically, the study of the importance of particular species has been analysed in the context of species interactions and the recognition that certain 'keystone species' (*sensu* Paine 1969) or 'ecosystem engineers' (*sensu* Jones, Lawton, & Shachak 1997) may have a disproportionate influence on entire communities or ecosystems (Mills, Soule, & Doak 1993; Hastings *et al*. 2007). Other studies have emphasized the role of a species as a 'conduit for energy and materials' (Hurlbert 1997), and this perspective reflects the recent attention on the contribution of individual species and overall biodiversity to ecosystem services (e.g., Spehn *et al*. 2002; Hooper *et al*. 2005; Zavaleta *et al*. 2010). A variety of measures have been

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proposed for quantifying species importance through the use of per capita effects (Power *et al.* 1996; Novak & Wootton 2010) and measures of unique function or contribution that cannot be provided by other species (Perry 2010).

The most straightforward way to measure species effects is through the experimental removal or addition of species to a community. However, such experiments may not be feasible, ethical or practical for many habitats and assemblages. Here, we develop a simple randomization test and an index of the importance of species to ecosystem processes that formalizes the analysis of a 'natural experiment' (*sensu* Cody 1974): a statistical comparison of measured ecosystem variables in unmanipulated samples with and without a particular species. We illustrated the test with measures of ecosystem function for biological crust communities, which are dominated by mosses, lichens, cyanobacteria and liverworts (Fig. 1), and play key ecosystem roles in arid and semi-arid habitats worldwide (Belnap & Lange 2003). The test identified particular species that showed strong positive or negative associations with ecosystem variables (e.g. biomass production, nitrogen retention, decomposition rate, soil moisture). We successfully validated the method through comparison with experimental microcosm data in which some of the same species were assembled in different combinations and the same ecosystem response variables were measured.

Methods

DATA INPUTS

The analysis uses two sets of data. First, we construct a species × sample binary presence–absence matrix, in which each row (i = 1 to S) represents a different species, and each column (j = 1 to N) represents a different sample. The entry x_{ij} is the presence (1) or absence (0) of species i in sample j. Second, we construct a vector of measurements of an environmental or ecosystem variable, with one measurement (or average) per sample (j = 1 to N measurements). The working hypothesis is that the presence or absence of a particular species has a significant effect on the environmental/ecosystem variable considered. The null hypothesis is that differences in such a variable measured in samples with and without a particular species are no greater than expected by chance. The method can also be extended to the analysis of abundance (or percentage cover) matrices by modifying the test metric to be the slope of the relationship between abundance and the measured environmental/ecosystem variable. A linear model with abundance as a predictor variable assumes a constant per capita contribution of each individual to the measured ecosystem variable. Nonlinear responses might be modelled with other kinds of trend analyses.

We assume that the direction of cause and effect is that species occurrences affect environmental variables. Alternatively, the analysis could be used to test whether environmental variables affect species occurrences, although the correct framework for that kind of analysis would be a logistic regression of species presence or absence versus the continuous environmental variable (see discussion).

A DIFFERENCE METRIC OF SPECIES IMPORTANCE

We define a simple difference metric D_i for the effect of species i on the environmental variable:

$$D_i = \bar{P}_i - \bar{A}_i$$

where $\bar{P}_i$ is the average of the environmental variable where species i is present, $\bar{A}_i$ is the average of the environmental variable where species i is absent and D_i is the difference between these two averages. Figure 2 illustrates the calculation of D_i values for a hypothetical data set with presence–absence data and a calculation of regression slopes for an analysis of abundance data.

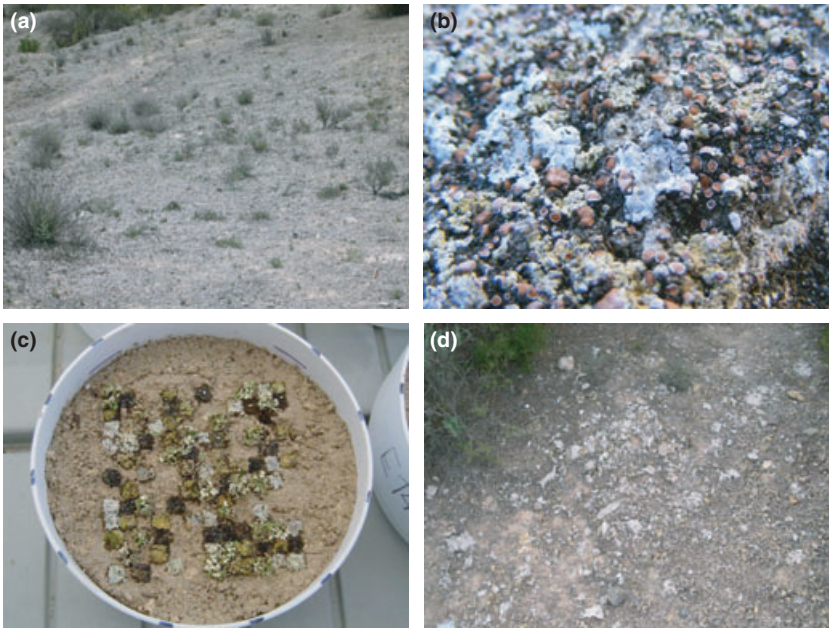

Fig. 1. Partial view of the semi-arid habitat in central Spain where the field data were gathered, (a); Close-up view of the biological soil crust community sampled (b); Experimental microcosm in which each species was introduced as a 1 cm^2 square of crust (c); Detail of *Diploschistes diacapsis* thalli loosely attached to the soil surface (d).

Species/Sample	A	B	C	D	E	F	G	H	I	J	K
S1	0	2	0	3	0	0	19	16	25	48	48
S2	0	0	0	6	0	0	0	0	0	0	0
S3	0	0	0	8	0	0	0	0	1	0	0
S4	24	5	4	5	3	0	1	10	10	10	7

Variable/Sample	A	B	C	D	E	F	G	H	I	J	K
Var1	0·095	0·485	0·049	0·376	0·183	0·023	0·935	0·631	0·426	0·777	0·685
Var2	0·284	1·374	1·648	1·728	2·95	0·068	1·005	1·892	1·279	2·331	2·055
Var3	0·568	2·748	3·296	3·456	5·899	0·136	2·011	3·784	2·558	4·663	4·109

	Presence - absence		Abundances	
		Var1		Var1
S1	Sum of presences/$n_{present}$	0·616	Regression slope	43·8
	Sum of absences/n_{absent}	0·088		
	Difference	0·528		

Fig. 2. Illustration of metric calculations for the randomization test. The sample matrix has four species (S1–S4) and 11 samples (A–K). The matrix entries represent the abundance (or percentage cover) of each species in each sample. For each sample, three ecosystem variables (Var1–Var3) are measured, and are illustrated in the second matrix. For the presence–absence analysis, we calculate the average of Variable 1 (0·616) for the samples that contained Species 1 (samples B, D, G, H, I, J, and K), and we calculate the average of Variable 1 (0·088) for the samples that did not contain Species 1 (samples A, C, E, and F). The difference between these two (0·528) is the test metric that is compared to the randomizations. For the % cover analysis, we calculated the slope (43·8) of a simple linear regression of Variable 1 on the abundance of Species 1 in all samples.

SIMULATION PROCEDURE

For a large number of iterations (typically 1000), the values of the environmental variable are randomly re-assigned to the different sites. With N samples, there are $N!$ unique, equiprobable re-arrangements of the vector that are possible. Therefore, for adequate statistical power, the test should not be used with fewer than seven samples (6! = only 720 unique arrangements). A bootstrapping procedure could also be implemented, in which observations are resampled multiple times from the vector of the environmental variable (Manly 2006). However, for the modest sample sizes in these kinds of analyses (often <100 samples), we prefer a simple re-assignment of the observed values to the different samples (sampling without replacement), which should minimize the effects of influential observations when sample size is small.

Note that the presence–absence matrix itself is not randomized, only the vector of the environmental variable. Thus, patterns of species co-occurrence and covariation are thus preserved in the assessment of species importance. This choice to randomize only the environmental variable reflects both the nature of the hypothesis being tested (species occurrences affect environmental variables, and not vice versa) and the fact that a variety of algorithms are possible for randomizing presence–absence matrices (Gotelli 2000), some of which may not be appropriate for measuring species importance. After each randomization, D_i values are calculated for each species.

STANDARDIZED INDEX

To measure species importance, we calculate a standardized effect size for each species (SES_i) as:

$$SES_i = \frac{D_i - D_{i(sim)}}{\sigma_{i(sim)}}$$

where D_i is the observed difference for species i, $\bar{D}_{i(sim)}$ is the average difference in the simulated data set and $\sigma_{i(sim)}$ is the sample standard deviation of the differences in the simulated data set. This index is similar to Power *et al.*'s (1996) estimate of community importance, except that it does not standardize for per capita effects, and it quantifies importance relative to the distribution of difference values in randomized data.

This SES index is derived from procedures in meta-analyses, where it is used to quantify effect sizes in comparisons of different treatments (Gurevitch *et al.* 1992). This index is also used in null model analysis to quantify the extent to which an observed metric deviates from the distribution of metrics generated by a stochastic null model simulation (Gotelli & McCabe 2002). This measure of effect size is the number of standard deviation units that the observed D_i lies above or below the expectation of the simulated distribution. If $|SES_i| > 2{\cdot}0$, then the observed value is approximately in the 5% tail of a normal distribution. If $|SES_i| < 2{\cdot}0$, the observed value is approximately within the range expected by chance. The assumption of a normal distribution of SES_i values has been validated in previous studies of effect size in null model analyses (Gotelli & Ulrich 2010; Ulrich & Gotelli 2010). Because species occurrences are not permuted, if two species have identical presence–absence sequences across sites, their measured effect sizes will be exactly the same.

The parametric analogue to our randomization test would be a simple t test for each ecosystem variable, comparing plots with and without a particular species. However, the parametric test assumes the ecosystem variables have a normal distribution. To examine the validity of this assumption, we also calculated two-sample t tests with unequal variances for the biological soil crust (BSC) Natural Experiment (see description below). For each t value, we calculated the standardized deviate, for direct comparison with our SES index from the randomizations.

We implemented our randomization tests in a Fortran 95 programme. Code and manual are given in Appendices S1 and S2. The software is posted on the webpage of WU (http://www.umk.pl/~ulrichw).

EMPIRICAL CASE STUDY: ECOSYSTEM FUNCTIONING IN BIOLOGICAL SOIL CRUST-FORMING LICHEN COMMUNITIES

We tested and validated our approach using BSCs dominated by mosses and lichens as a model system. These organisms have a great impact on ecosystem functioning: They control water fluxes such as infiltration and runoff (Belnap 2006; Eldridge *et al.* 2010), stabilize the soil surface (Belnap & Gillete 1998) and influence the cycles of carbon (Maestre & Cortina 2003; Thomas, Hoon, & Linton 2008) and nitrogen (Belnap 2002; Castillo-Monroy *et al.* 2010; Delgado-Baquerizo *et al.* 2010). These functional roles of BSCs, together with their small size, make them a useful model system to explore the relationships between species occurrence and ecosystem functioning

(Bowker, Maestre, & Escolar 2010; Eldridge *et al.* 2010; Maestre *et al.* 2010).

For this analysis, we used data from a natural experiment (occurrences of species in unmanipulated plots) and a controlled field experiment (experimental assembly of microcosms with different species composition) and measured the same suite of environmental/ecosystem variables in both the natural experiment and the microcosm experiment.

BSC NATURAL EXPERIMENT

Field data on BSC abundance and ecosystem functioning were obtained from Maestre *et al.* (2008, 2010). Data were gathered in semi-arid (mean annual temperature and rainfall of 14 °C and 452 mm, respectively) gypsum outcrops located next to Belmonte del Tajo, in Central Spain (40° 7′ 3″N, 3° 18′ 30″W, 686 m a.s.l.). The studied outcrops support a very low perennial vascular plant cover (< 20% of the total surface area, Fig. 1a) and have a prominent BSC community dominated by the lichens *Diploschistes diacapsis* (Ach.) Lumbsch, *Acarospora nodulosa* (Dufour) Hue, *Cladonia convoluta* (Lam.) Anders and *Collema crispum* (Huds.) F. H. Wiggr (see Maestre *et al.* 2008 for details; Fig. 1b). A total of 63 plots (50 cm × 50 cm), within a homogeneous area of 1·3 ha, were established in patches with well-developed BSC-forming lichen communities and with almost no vascular plants (cover < 5% in all plots). These assemblages were dominated by lichens, with < 10% cover by mosses. A minimum distance of 0·7 m between sampling plots was established to ensure statistical independence of the samples. Although the survey aimed to capture the range of variation in BSC communities, all plots were of the same general habitat type. In the absence of species interactions or dispersal constraints, it would not be surprising to encounter any of the species in a particular plot.

Each plot was divided into hundred 5 cm × 5 cm sampling quadrates, and the percentage cover of every lichen species was estimated in all quadrates. Field surveys were carried out during the winter of 2005 and the spring of 2006. The average cover in the 100 quadrates was used as an estimate of the cover of each species per plot. A total of 17 species were recorded in the 63 plots. One species, *D. diacapsis*, was present in every plot (Fig. 1d), so it could not be used in presence–absence analyses (no absences were found), but it was used in the analysis of percentage cover, which did vary among the plots.

BSC MICROCOSM EXPERIMENT

We compared the results of the analysis of the natural experiment with data from a microcosm manipulative experiment (F. T. Maestre & A. Castillo-Monroy, unpublished data). This experiment was conducted in the facilities of the Rey Juan Carlos University, located in Móstoles (Central Spain, 620 m a.s.l.). Soil and BSC-forming lichen species were collected from gypsum outcrops located over 50 km south of the university.

The basic experimental unit was a microcosm built from PVC pipe (length 8 cm, internal diameter 20 cm) filled with 7 cm of field soil. This soil was thoroughly mixed and homogenized with a cement mixer before filling the microcosms. To check for homogeneity of the substrate, we analysed two soil samples from different parts of the soil pile at the start of the experiments. For all ecosystem variables, measured differences between the two samples were < 5%. Thus, we assumed that the initial soil conditions were homogeneous for all the microcosms. Intact lichen pieces were collected from the field, separated into species and cut into homogeneous 0·5-cm-side square fragments (Fig 1c). These fragments were added to the surface to achieve a 60% coverage of each microcosm unit, which is within the range found in the field (39–98%, Maestre *et al.* 2005). The experiment was designed to independently test for the effects of species richness, species composition and spatial pattern on ecosystem functioning.

Four unique species composition treatments were established by random sampling from a pool of 10 common BSC-forming lichen species [*A. nodulosa*, *Collema crispum*, *D. diacapsis*, *Fulgensia subbracteata* (Nyl.) Poelt, *Lepraria crassissima* (Hue) Lettau, *Psora decipiens* (Hedw.) Hoffm., *Psora saviczii* (Tomin) Follmann and A. Crespo, *Squamarina cartilaginea* (With.) P. James, *Squamarina lentigera* (Weber) Poelt and *Toninia sedifolia* (Scop.) Timdal]. Species combinations were nested within two species richness levels (four and eight species, Table 1). Each combination of species composition and richness was established under two spatial patterns: clumped and random. The cover of each lichen species in the four- and eight-species mixtures was 15% and 7·5%, respectively. Thus, total lichen cover across microcosms was held constant (60%). Each combination of richness (2), composition (4) and spatial pattern (2) was replicated six times for a total of $2 \times 4 \times 2 \times 6 = 96$ microcosms. Control microcosms (containing only soil) were also setup. The experiment was conducted under natural light, temperature and rainfall conditions between June 2006 and December 2008.

BSC ENVIRONMENTAL/ECOSYSTEM VARIABLES

In both the natural experiment and the microcosm experiment, we measured the following soil variables: organic carbon, total nitrogen and the activity of three enzymes related to the carbon (β-glucosidase), nitrogen (urease) and phosphorus (phosphatase) cycles. These variables are good indicators of nutrient cycling, a critical determinant of the functioning of arid and semi-arid ecosystems (Whitford 2002).

Soil sampling was conducted in the natural experiment in late September 2006. Twelve randomly placed 19·63-cm^2 circular soil cores (5 cm diameter, ×1 cm depth) were collected from each plot, and bulked and homogenized in the field. The microcosms were harvested at the end of the experiment, in December 2008. During the harvest-

Table 1. Different composition levels used in the microcosm experiment conducted with biological soil crust-forming lichens

Composition number	Species included
1	Cc, Sl, Co, Fs
2	Ts, Dd, An, Pd
3	Sc, Dd, An, Sl
4	Ts, Pd, Dd, Sl
5	Cc, Co, An, Pd, Fs, Sc, Sl, Dd
6	Ts, Cc, Lc, Pd, Fs, Sc, Sl, Dd
7	Ts, Cc, Co, Lc, Pd, Fs, Sl, Dd
8	Ts, Co, An, Lc, Pd, Fs, Sc, Dd

An, *Acarospora nodulosa* (Dufour) Hue; Cc, *Cladonia convoluta* (Lam.) Anders; Co, *Collema crispum* (Huds.) F. H. Wiggr; Dd, *Diploschistes diacapsis* (Ach.) Lumbsch; Fs, *Fulgensia subbracteata* (Nyl.) Poelt; Lc, *Lepraria crassissima* (Hue) Lettau; Pd, *Psora decipiens* (Hedw.) Hoffm.; Sc, *Squamarina cartilaginea* (With.) P. James; Sl, *Squamarina lentigera* (Weber) Poelt; Ts, *Toninia sedifolia* (Scop.) Timdal.
The composition numbers correspond to the treatment levels in Fig. 3.

ing of the microcosms, a composite sample of the soil from all areas of the microcosm covered by lichens (60% of the surface) was obtained for the 0–2 and 2–5 cm depths; only the former depth is used here. In both the natural and microcosm experiments, the lichens were carefully removed with a knife to avoid measuring those nutrients incorporated in or adherent to them, and soil samples were air-dried for a month in the laboratory prior to analyses. Total N was obtained using a SKALAR San + + Analyzer (Skalar, Breda, The Netherlands) after digestion of the soil samples with sulphuric acid. Urease activity was determined as the amount of NH_4^+ released from 0·5 g soil after incubation for 90 min with urea (6·4%) at 30 °C in phosphate buffer (pH 7; Nannipieri *et al.* 1980). Phosphatase activity was measured by determination of the amount of p-nitrophenol (PNF) released from 0·5 g soil after incubation at 37 °C for 1 h with the substrate p-nitrophenyl phosphate in Modified Universal Buffer (MUB) buffer (pH 6·5; Tabatabai & Bremner 1969). The activity of β-glucosidase was assayed according to Tabatabai (1982), following the procedure for phosphatase, but using p-nitrophenyl-β-D-glucopyranoside as substrate and Trishydroxymethyl aminomethane instead of NaOH. Soil organic carbon was estimated using the Walkley-Black method (Nelson & Sommers 1982).

Some mortality of transplanted lichens occurred during the experiment (A. P. Castillo-Monroy and F. T. Maestre, unpublished data), and thus, the species composition at sampling time departed from the initial composition in some microcosms. No new species of lichen colonized the mesocosms during the experiment. However, we did not conduct a frequent and repeated monitoring of survival during the experiment, and thus, we do not know the exact date when mortality occurred. Therefore, we analysed the results using the initial planted composition.

STATISTICAL COMPARISONS OF BSC NATURAL EXPERIMENT AND MICROCOSM EXPERIMENT

In the BSC microcosm experiment, any differences in measured ecosystem variables can be attributed to differences in species composition, which was manipulated directly. Therefore, these data provide a valuable test of the species importance index, which was calculated for the survey data. However, the microcosm experiment was not designed to test our statistical method, and it is not strictly analogous to the 'natural experiment' that is implied by the randomization test. Therefore, it was necessary to modify the analysis of the microcosm data for comparison with the randomization test.

We first used a simple one-way ANOVA to assay whether there were differences among the eight experimental treatments (which differed in both species richness and composition) in each ecosystem variable. Because the spatial pattern in real communities is often intermediate between the purely random and highly clumped designs used in the microcosm experiment (Maestre *et al.* 2005), we ignored spatial arrangement as a factor in this one-way ANOVA (eight assemblages × 12 replicates = 96 microcosms). Of the five ecosystem variables measured, organic carbon and total nitrogen differed significantly among the eight composition treatments (organic carbon: $F_{7,88} = 7{\cdot}31$, $P < 0{\cdot}001$; total nitrogen: $F_{7,88} = 3{\cdot}17$, $P = 0{\cdot}005$). Results were similar for a more complicated split plot ANOVA that also tested for effects of spatial pattern, species composition and species richness. ANOVA analyses were carried out using SPSS version 15.0 (Norušis 2007).

For each treatment, we next calculated, from the presence–absence analysis of the natural experiment (Table 2), the average SES of organic carbon (or total nitrogen) for all the species that were initially represented in each microcosm treatment. We then regressed organic C and total N for each microcosm treatment against the average SES calculated for that species composition. If the SES indices from the survey data reflect the additive contribution of different species to measured organic C or total N, these two measures (derived independently from survey and experimental data) should be significantly correlated.

Results

NATURAL EXPERIMENT

Table 2 summarizes the results for the randomization tests of each of the 16 species with each of the five ecosystem variables. Results are given for both presence–absence analysis and percentage cover analysis. For the presence–absence analysis, of the $16 \times 5 = 80$ tests, 13 gave a significant positive result (higher levels of the ecosystem variable when the species was present) and six gave a negative result (higher level of the ecosystem variable when the species was absent). If the tests were all random and independent, there should have been a total of only ~4 significant values in the first ten columns of Table 2 (5% of 80). Results were similar, but not identical, for the percentage cover analysis.

For the presence–absence analysis, 11 of the 13 positive responses were for urease activity, and five of the six negative responses were for β-glucosidase activity. Organic carbon exhibited one positive [*Placidium pilosellum* (Breuss) Breuss] and one negative [*Lepraria crassissima* (Hue) Lettau] species response, and total nitrogen exhibited one positive species response (*P. pilosellum*). None of the 16 species exhibited a significant effect on phosphatase activity. The presence or absence of four species [*A. nodulosa, C. convoluta, S. lentigera, Toninia toniniana* (A. Massal) Zahlbr] had no measurable effects on any of the ecosystem variables, although each of these species had significant effects on one ecosystem variable when the data were analysed as percentage cover. Positive and negative effects were split approximately evenly, except for urease activity, in which there was a positive response to the presence of all 16 species.

The frequency of statistically significant effects differed between the randomization tests and the *t* tests. Whereas the randomization tests revealed 13 positive and six negative responses, the *t* test revealed 16 positive and 15 negative responses. The results were sensitive to the particular ecosystem variable used. For example, the randomization test revealed no significant effects of species occurrence on soil pH, whereas the *t* tests revealed six negative and two positive effects (Table 2).

COMPARISON OF FIELD AND MICROCOSM EXPERIMENTS

For both total nitrogen and organic carbon, measured values in the microcosm experiment differed significantly among species composition treatments (Fig. 3, upper panels). For each of these variables, the microcosm measures were significantly correlated with the average SES for the species composition represented in each treatment (Fig. 3, lower panels). Thus, species combinations that had high levels of nitrogen or organic car-

Table 2. SES values and *t* scores obtained for each species in the field survey, using presence–absence and % cover data. Species with underlined names were used in the mesocosm experiment (Table 1). Significant positive and negative *Z* values and *t* scores are indicated by dark pink (SES ≥ 2·0) and light blue shades (SES ≤ −2·0), respectively

	Presence–absence					% Cover					*t* scores				
Species	OC	TN	UR	PH	BG	OC	TN	UR	PH	BG	OC	TN	UR	PH	BG
Acarospora nodulosa	0·62	0·97	1·33	−1·60	0·34	−0·93	−1·27	1·60	−0·64	−2·36	1·79	2·29	2·74	−0·84	0·69
Placidium pilosellum	2·19	2·21	2·86	−0·71	−0·31	2·96	2·98	0·99	−1·81	0·25	0·11	1·54	2·89	−1·77	−0·28
Placidium squamulosum	1·36	1·57	2·14	−0·84	−0·84	1·61	1·15	2·27	0·18	0·75	1·66	1·85	2·41	−2·53	−0·89
Cladonia convoluta	1·67	0·93	1·91	1·13	1·24	1·30	1·92	3·40	−0·18	0·71	0·44	0·50	1·76	0·43	0·52
Collema crispum	−1·51	−0·87	2·37	−0·72	−3·75	0·80	0·54	3·05	−0·98	−1·83	−2·90	−1·04	3·71	−3·98	−3·87
*Diploschistes diacapsis**	NA	NA	NA	NA	NA	−0·27	−0·48	−1·49	0·57	1·47	NA	NA	NA	NA	NA
Endocarpon pusillum	−1·88	−1·08	3·71	1·23	−1·69	0·01	0·11	3·42	0·44	−0·84	−2·48	−0·60	3·66	2·35	−1·73
Fulgensia subbracteata	−1·21	−0·95	3·05	−0·39	−4·12	−3·43	−2·57	1·79	−0·42	−4·43	−3·54	−1·20	4·97	−5·75	−4·71
Lepraria crassissima	−2·02	−1·44	0·18	1·46	0·97	−0·21	0·42	2·15	1·27	0·60	−1·55	−0·95	0·16	0·77	1·03
Psora globifera	−1·38	−0·55	3·01	0·90	−1·99	−1·33	−0·91	1·08	1·45	−1·21	−5·20	−1·50	3·01	2·28	−2·08
Psora decipiens	−0·16	0·22	2·56	−0·81	−3·75	0·70	0·39	3·73	−0·57	−1·98	−3·14	0·67	4·27	−5·27	−4·03
Psora saviczii	1·14	0·11	2·70	−0·56	−2·60	−0·23	0·15	2·90	−1·23	−1·79	0·71	0·15	3·74	−1·94	−0·37
Squamarina cartilaginea	−0·26	0·74	2·55	−0·40	−0·54	0·05	0·16	4·96	1·52	−0·77	−0·71	0·15	3·74	−1·94	−0·37
Squamarina lentigera	1·59	1·56	0·45	−1·62	−1·28	0·58	0·40	2·61	−1·02	−2·02	2·56	0·76	0·68	−3·76	−0·76
Toninia albilabra	−1·09	−0·56	3·71	−0·94	0·10	1·24	1·29	1·84	−0·62	0·34	−1·49	−1·43	3·02	−2·34	0·12
Toninia sedifolia	1·24	1·38	2·17	−0·34	−2·35	−0·45	−0·29	2·19	−0·01	−1·92	3·13	0·52	2·00	−3·33	−2·49
Toninia toniniana	0·62	0·97	1·45	−1·43	0·79	1·29	1·60	2·02	−1·01	1·14	0·34	0·84	0·88	−0·53	0·64

OC, organic carbon; TN, total nitrogen; UR, urease activity; PH, phosphatase activity; BG, β-glucosidase activity; SES, standardized effect size.

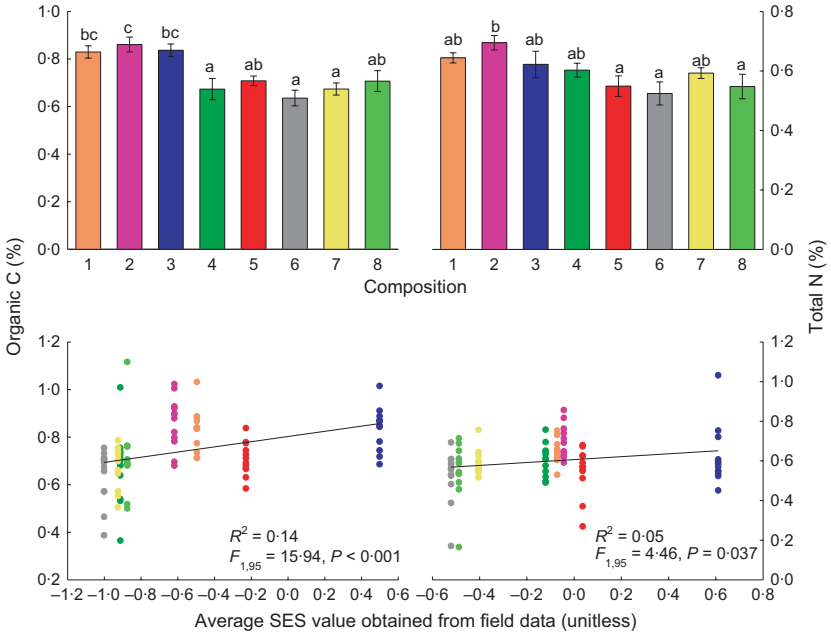

Fig. 3. Effects of species composition on Organic carbon (C) and total nitrogen (N) observed in the microcosm experiment (upper panels), and the correlation between species importance calculated from the survey data and C (lower left panel) and N (lower right panel) in the microcosm experiment. In the upper panels, each bar is the average C or N for the eight species combinations listed in Table 1 (control microcosms without lichens are not shown for these comparisons). Compositions 1–4 and 5–8 correspond to assemblages with four and eight species, respectively. The vertical lines in the upper panel represent 1 standard deviation (n = 12 replicates). Different lowercase letters above the bars indicate significant differences among composition levels after One-way ANOVA (Tukey HSD *post-hoc* test, P < 0·05). In the lower panels, each point represents the measured absolute value of C or N for an individual replicate. On the x axis, the average SES from the field survey (Table 2) was calculated for the species composition represented by that treatment in the microcosm experiment.

bon contained species that were also associated with higher levels of these variables in the survey data (Table 2).

Discussion

Although many other methods exist for quantifying species importance, the randomization test we propose has several advantages. First, it formalizes and makes explicit the 'natural experiment' philosophy (Diamond 1986), which is to take advantage of natural variation in species composition and measure differences in ecosystem function that are associated with this variation. The test provides a simple statistical assay of the pattern and can function well with the small to moderate sample sizes that ecologists usually have for field data. It can be applied to both experimental and survey data, and our initial analysis reveals that some of the patterns were corroborated in independent experimental manipulations (Fig. 3). Our randomization test is analogous to a parametric *t* test, but *t* tests assume data are normally distributed; the results may be sensitive to outliers and unbalanced sample sizes. In our comparison of these two methods, the randomization test gave more conservative results (Table 2). Many of the significant *t* test results in Table 2 (especially for pH) probably reflect violation of the model assumptions, rather than true species effects.

Perhaps, the most important difference between our index and most previous measures is that we have chosen not to scale the effects on a per capita basis. There are three reasons for this as follows: (i) scaling the results by abundance or biomass means that the ecosystem measurements for rare species would be divided by a very small number, which could greatly inflate the errors and uncertainty in the index; (ii) for many kinds of species – including BSCs – modular organisms grow as colonies or clones for which it is not possible to recognize a single individual (Fig. 1b); (iii) total biomass or abundance is itself an important species attribute that certainly contributes to the net effect of a species on ecosystem variables (e.g. Maestre *et al.* 2005, 2010).

Microcosms that contained only soil had lower organic C and total N values than measured in any of the experimental species assemblages (C = 0·62% ± 0·07; N = 0·41% ± 0·01, means ± SE, $n = 12$), indicating that lichens collectively increased C and N over the course of the experiment. Interestingly, in contrast to other semi-arid environments (Belnap 2002), N levels were apparently unrelated to the presence of the only nitrogen-fixing species studied, the lichen *C. crispum*. In the microcosm experiment, assemblages containing this species (treatments 1, 5, 6 and 7 in Fig. 3) did not generate higher N values. In the field survey, the SES values for the effect of *C. crispum* on N were either weakly negative (SES = −0·87; presence–absence data) or weakly positive (SES = 0·54; cover data). These results are consistent with other measurements from BSC communities in central and south-eastern Spain (Maestre *et al.* 2005). Collectively, these results suggest that other BSC constituents that are able to fix nitrogen, such as free-living bacteria and cyanobacteria, could provide such inputs to the soil (Zaady, Groffman, & Shachak 1998). Cyanobacteria and free-living bacteria commonly grow epiphytically on soil mosses and lichens (DeLuca *et al.* 2002; Belnap & Lange 2003), and their abundance and activity patterns are often linked to particular moss and lichen species (Redfield *et al.* 2002; Hu *et al.* 2003).

If all the individuals of different species made equal contributions to ecosystem function, then the most abundant species would exhibit the greatest contribution. At our field site, the most abundant species was *D. diacapsis*, which was present in every plot and had an average cover higher than 70% (Maestre *et al.* 2008). Interestingly, variation in percentage cover of this species was not significantly associated with differences in any ecosystem variable (Table 2). *Diploschistes diacapsis* is often loosely attached to the soil (Fig. 1d), which may weaken its effects on some soil properties evaluated. When it is detached from the soil surface, this species decreases infiltration and promotes runoff (Souza-Egipsy, Ascaso, & Sancho 2002), which might also reduce microbial activity associated with carbon and nutrient cycling because of reduced soil moisture beneath this species (Austin *et al.* 2004; Castillo-Monroy *et al.* 2010). In the microcosm experiment, only the first species combination was lacking *D. diacapsis*, but this treatment had intermediate levels of total N and organic C (Fig. 3, upper panel), which is consistent with the SES values in the survey data (Table 2). Overall, measured SES values in the field survey were uncorrelated with abundance ($r < |0{\cdot}33|$, $P > 0{\cdot}20$ in all cases), which is consistent with the studies in which strong community effects are not necessarily related to total abundance (Paine 1992).

In this study, we had the unique opportunity to validate SES measures by comparing them to the results of an independent manipulative experiment. Both organic carbon and total nitrogen varied significantly as a function of species combination (Fig 3, upper panels), and in both cases, those ecosystem variables were significantly correlated with an index based on the SES values from the field survey (Fig 3, lower panels). However, the microcosm experiment was not designed to test the response of individual species, so the results have to be interpreted carefully. In the microcosm experiment, the presence of each species was not varied one at a time. Instead, entire sets of species were manipulated to produce eight assemblage types that varied in species richness and composition. As a consequence, for variables such as urease activity, in which there were many strong positive responses in the survey data (Table 2), there may not have been sufficient variation between the experimental assemblages for a valid regression analysis. Also, in the microcosm experiment, differences in microhabitat were eliminated through the use of a common source of sifted soil. Responses of ecosystem variables and species occurrences to natural variation in microhabitat may have contributed to some of the significant results in the randomization tests (Table 2).

Nevertheless, there are some potentially important weaknesses in our analysis, which assumes independent additive contributions of species to measured variables. Our estimate of species effects on ecosystem function could be biased if: (i) strong species interactions control the distribution of species (Bowker, Soliveres, & Maestre 2010) or

mediate the response to ecosystem variables (Kikvidze *et al.* 2005); (ii) species occurrences are correlated with unmeasured abiotic variables that also affect ecosystem function (Maestre *et al.* 2009); such correlations may be magnified by underlying spatial autocorrelation in environmental variables and species occurrences; (iii) the direction of cause and effect is reversed, and it is actually ecosystem variables that are affecting the commonness and rarity of species (e.g. Baer *et al.* 2004); if this is the hypothesis, the randomization test can be modified to assign species occurrences randomly and independently to sites that differ in a measured environmental variable; (iv) effects of species occurrences on ecosystem variables are nonlinear or unimodal. In this case, our test could be modified to calculate the sample variance, rather than the mean, of the response variable in sites with and without a particular species. If responses are unimodal, then variances of the two groups will differ widely.

An additional complication with the randomization test is that it treats the contribution of each species in isolation, whereas the actual response of the ecosystem variable should reflect the additive effects of all species in the plot (D. Faith, pers. comm.). To explore this idea with per cent cover data, we used a linear multiple regression model in which the response was the ecosystem variable (total nitrogen or organic carbon), and the predictor variables were the per cent cover measures for all 17 species. We then used the t value of each coefficient from the regression model as the analogous score of species importance for comparing with the SES from the randomization test (both the SES and the t value measure the extent to which the observed data deviate from the null distribution). For both analyses, the two metrics were strongly correlated (organic carbon: $r^2 = 0{\cdot}67$, $P < 0{\cdot}001$; total nitrogen: $r^2 = 0{\cdot}68$, $P < 0{\cdot}001$). For total nitrogen, both analyses identified the same two species as statistically significant, with similar positive and negative effect sizes. For organic carbon, the regression model identified an additional two species with positive effects that were not detected by the randomization test. However, the significance tests for the regression model depend on assumptions of linearity, constant error variances and lack of error in the measurement of the predictor variable (Gotelli & Ellison 2004). These assumptions will not always be met in the studies of ecosystem response variables. Because the rank order of the coefficients in the two analyses was similar, the results suggest that the randomization test for these data (which assumes independent effects of each species) was not seriously distorted by the additive contributions of multiple species to ecosystem variable responses (which were measured with the regression analysis).

However, complicating factors such as additive species effects are not specific to the test that we propose or to the BSC community in central Spain. Rather, they are potential weaknesses of any natural experiment in which mechanisms are inferred from patterns of uncontrolled variation in nature (McGuinness 1988). One pedagogical advantage of using randomization tests is that they make the underlying assumptions explicit and very clear. When ecologists rely on familiar parametric tests, some of these assumptions and limitations may not be so obvious.

Despite the potential limitations of our approach, its successful validation suggests that simple randomization tests may be useful for exploring associations of species and ecosystem variables. Further, insight into the mechanisms responsible for these patterns can be gained from experimental removal or addition of individual species and from comparisons of ecosystem properties in experimental monocultures and polycultures (Potvin & Gotelli 2008).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. User's manual for Impact software.

Appendix S2. *Impact* – a FORTRAN program for gradient analysis.

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