

Initial community evenness favours functionality under selective stress

Lieven Wittebolle^{1*}, Massimo Marzorati^{1*}, Lieven Clement², Annalisa Balloi⁴, Daniele Daffonchio⁴, Kim Heylen³, Paul De Vos³, Willy Verstraete¹ & Nico Boon¹

Owing to the present global biodiversity crisis, the biodiversity–stability relationship and the effect of biodiversity on ecosystem functioning have become major topics in ecology^{1–3}. Biodiversity is a complex term that includes taxonomic, functional, spatial and temporal aspects of organismic diversity, with species richness (the number of species) and evenness (the relative abundance of species) considered among the most important measures^{4,5}. With few exceptions (see, for example, ref. 6), the majority of studies of biodiversity–functioning and biodiversity–stability theory have predominantly examined richness^{7–11}. Here we show, using microbial microcosms, that initial community evenness is a key factor in preserving the functional stability of an ecosystem. Using experimental manipulations of both richness and initial evenness in microcosms with denitrifying bacterial communities, we found that the stability of the net ecosystem denitrification in the face of salinity stress was strongly influenced by the initial evenness of the community. Therefore, when communities are highly uneven, or there is extreme dominance by one or a few species, their functioning is less resistant to environmental stress. Further unravelling how evenness influences ecosystem processes in natural and humanized environments constitutes a major future conceptual challenge.

Several components of biodiversity, such as species and functional group richness, have been shown to influence ecosystem function and stability significantly^{3,12}. Species evenness has similarly been shown to influence community dynamics¹³ and be an important element in managing invasions and production in managed ecosystems^{14–16}. However, the influence of species evenness on the stability of ecosystem functioning remains unknown. Theoretically, evenness could strongly influence the stability of ecosystem functioning. For example, in a community where species are functionally redundant (that is, most contribute to the ecosystem function of interest), if initial evenness is high then the probability that a species tolerant to a perturbation is present is higher than when evenness is low. When evenness is low, meaning that the community is dominated by one or a few species, resistance to the perturbation will only occur if the dominant species are tolerant to the perturbation.

To test the relationship between initial community evenness and functionality, we used microcosm tests with denitrifying bacterial model communities. These are tools well suited to addressing ecological questions, as they can be maintained under simplified and defined conditions^{17–19}. In addition, denitrifier models are good for investigating the value of microbial biodiversity in ecosystem functioning, owing to the wide range of physiological properties in this functional group of bacteria²⁰. Different levels of initial evenness were assembled by, in each mixture, using eighteen different denitrifying

species from four different phyla (Supplementary Table 3). We acknowledge that the degree of evenness will probably change during the course of the experiment. However, our hypothesis aims to test the response of the initial community evenness and how this translates itself into functional stability, regardless of any further shifts in community structure. A total of 1,260 microcosms, all with the same richness, were set up, incubated for 20 h under three distinct conditions (no stress, low temperature and salt stress), and related to the stability of the net ecosystem denitrification as a measure of the ecosystem functionality. All selected denitrifying species had similar activity response ranges, in order that all could contribute to ecosystem productivity. They represented an average range of richness broad enough to ensure good functionality⁷. Varying evenness without changing richness decreases the confounding of diversity by species identity^{2,5}.

Lorenz curves were used to assess initial community evenness visually. The Gini coefficient (ranging from zero to one) is a single value that describes a specific degree of evenness (Supplementary Fig. 3), measuring the normalized area between a given Lorenz curve and the perfect evenness line. The higher the Gini coefficient, the more uneven a community is. Lorenz curves of all 1,260 microcosms showed that almost the entire evenness range was sampled (Fig. 1). The net ecosystem denitrification of the investigated microbial

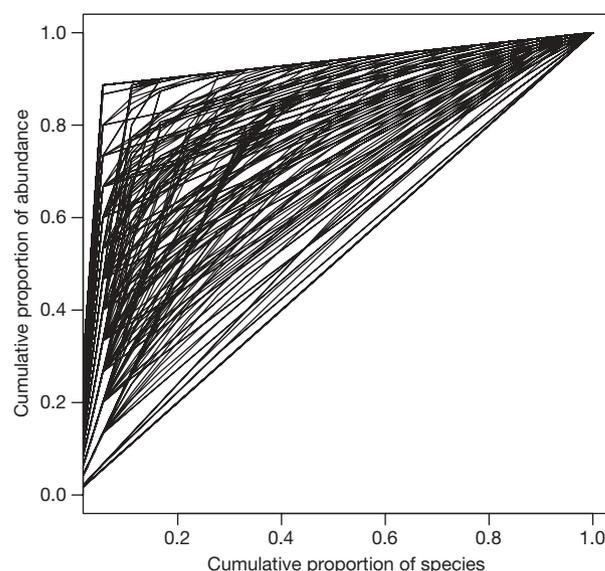


Figure 1 | Lorenz curves used in the experiment. The curves span the entire region between perfect evenness and high dominance.

¹LabMET, Laboratory of Microbial Ecology & Technology, ²BIOSTAT, Department of Applied Mathematics, Biometrics and Process Control, ³LM-UGent, Laboratory of Microbiology, Department of Biochemistry, Physiology and Microbiology, Ghent University, B-9000 Ghent, Belgium. ⁴DISTAM, Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Università degli Studi di Milano, 20133 Milan, Italy.

*These authors contributed equally to this work.

Table 1 | Linear models estimating the effects of various factors on the denitrification functionality

Step	Model	Residual d.f.	Residual SS	Treatment d.f.	Treatment SS	AIC
0	Intercept	1,439	29.06	—	—	-1,529.90
1	0 + P + R + C + B + I	1,398	21.43	41	7.63	-1,886.15
2	1 + S	1,396	13.44	2	7.99	-2,554.30
3	2 + S:P	1,388	10.25	8	3.19	-2,928.20
4	3 + S:I	1,352	7.96	36	2.29	-3,325.30
5	4 + G ² + S:G ²	1,349	7.14	3	0.82	-3,370.40
6	5 + B:S	1,347	6.65	2	0.49	-3,469.80
7	6 + S:R	1,333	6.46	14	0.19	-3,484.20
8	7 + S:C	1,311	6.24	22	0.22	-3,489.40
9	8 + X + S:X	1,308	6.23	3	0.01	-3,485.60

The linear models describe the effects of stress, *S*, identity of the dominant species, *I*, Gini coefficient, *G*, and the relative abundance of the dominant species, *X*, on the functionality. The models also allow corrections for experiment effects, *P*, row effects, *R*, column effects, *C*, and the negative controls, *B*. Interactions are indicated using colons. At each step (1–9), terms were added to the model. The residual degrees of freedom (d.f.) and sum of squares (SS) are given. The treatment degrees of freedom and sum of squares only apply to the term that was added to the model. The Akaike information criterion³⁰ (AIC) was calculated for each model; a lower AIC indicates an improved model.

communities was expressed by the difference between the nitrite concentration of the negative controls and the residual nitrite of each microcosm after incubation. Linear models were used to assess the effects of the stress, *S*, the Gini coefficient, *G*, the relative abundance of the dominant species, *X*, and that of their interactions on ecosystem functionality. Both *G* and *X* are shape parameters describing a particular Lorenz curve. In addition to the factors considered above, several confounding factors could be present. Potential row, *R*, column, *C*, and experiment, *P*, effects due to the multiwell analysis process, negative controls, *B*, and the identity of the dominant species, *I*, were taken into account to allow a correct estimation of the model parameters being studied. Model selection was performed using a series of linear models in which each of the effects and interactions were entered sequentially (Table 1). After including the confounding factors, terms that resulted in the largest decrease of the AIC were added to the model. On the basis of the AIC, model 8 was selected (coefficient of multiple correlation, $R^2 = 78.5\%$). A residual analysis showed that the model fit was adequate (Supplementary Fig. 4).

From an ecological perspective, the assessment of stress, the identity of the dominant species and the shape parameters *G* and *X* are of great importance. There was a very significant effect on ecological functioning due to stress (chi-squared test, $P < 0.001$) and the latter's interactions with other variables (chi-squared test, $P < 0.001$). Hence, the type of stress had a strong impact on the functionality and on the contribution of the other variables in the model. Moreover, there was a very significant interaction between the identity of the dominant species and the stress. Chi-squared tests indicated that dominant species identity had no effect in the control environment ($P = 0.22$) or in the temperature-stressed environment ($P = 0.10$). The parameter estimates and tests (Supplementary Fig. 5) both showed that temperature had a negative impact on functionality. By contrast, the identity of the dominant species was shown to be significant (chi-squared test, $P < 0.01$) in the case of salt stress.

This type of stress can therefore be considered selective, that is, one that disfavours some species but favours others (Supplementary Fig. 5). It should be noted that the functionality of none of the species was completely inhibited by temperature or salt stress (Supplementary Fig. 5).

The effect of the initial evenness on functionality and functional stability was modelled as a quadratic effect of the Gini coefficient (Fig. 2). The Gini coefficient was seen to have a very significant effect in both the control case and the salt-stress case ($P < 0.001$ for both tests). Both graphs (Figs 2a, c) show that functionality decreased with increasing initial unevenness and that this effect was more pronounced in the case of salt stress ($P < 0.001$). However, the adverse effect of initial unevenness can be partly overcome when the most dominant species is stress resistant, as illustrated by the interactions between the identity of the dominant species and salt stress. With regard to temperature, the degree of initial evenness had no significant effect, as growth was limited at low temperatures. Thus, in this situation, low temperature can be considered a severe, non-selective stress condition.

The type of stress had a distinct effect on the stress-buffering capability of a community (Fig. 3). A stress that disfavours all species to nearly the same extent decreases the functionality of the community regardless of its initial evenness. However, the degree of evenness is a key feature in cases of selective stress, which are the most frequent situations in nature^{5,21,22}. We found that, on average, initial community unevenness decreases the functional stability when selective stress is applied. Nevertheless, exceptions occur, such as when the dominant species of an uneven community is favoured by the stress. Notably, increased initial community unevenness also lowered the functionality of unstressed communities, albeit not to the same extent as under selective stress.

Past practical and theoretical constraints have limited the ability to relate patterns of microbial evenness with the processes that determine

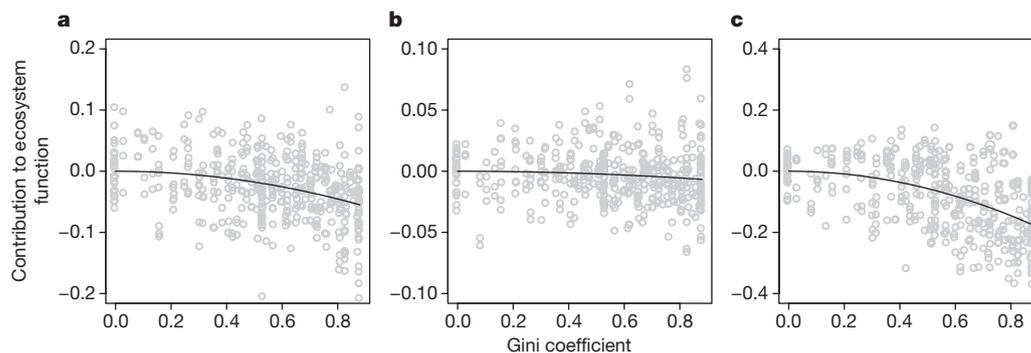


Figure 2 | Contribution of increasing initial unevenness (Gini coefficient) to the functionality of the ecosystem (that is, net denitrification after 20 h of incubation). **a**, No stress ($n = 420$); **b**, temperature stress ($n = 420$); **c**, salt stress ($n = 420$). This contribution to ecosystem function represents the effect of the Gini coefficient on the functionality corrected for row, column,

experiment, negative control and main effect for stress. Partial residuals (contribution of the Gini coefficient plus residual) are indicated by open circles. They illustrate the extent of uncertainty that could not be explained by the model.

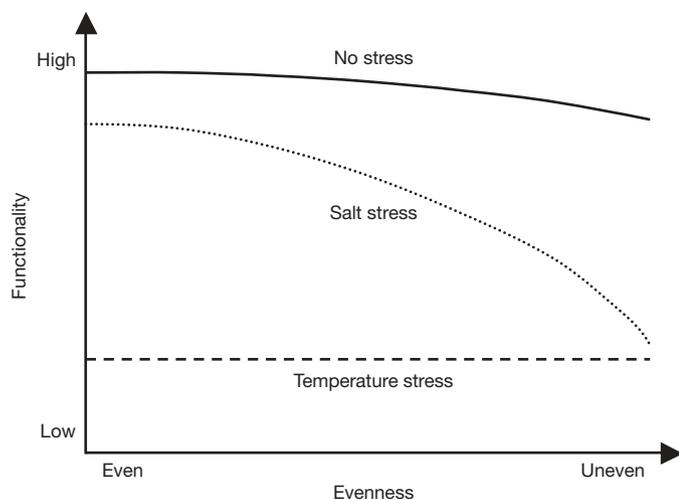


Figure 3 | Microbial functionality in relation to the initial evenness, for different types of stresses. The selective stressor NaCl has a much more negative impact on the functionality of the unevenly distributed microbial community.

these patterns. Nevertheless, recent studies have indicated that bacterial diversity may follow regular patterns, and that in some cases these patterns may be qualitatively similar to those observed for plants and animals²³. Decreases in evenness (for example as a response to environmental changes) may have an indirect lowering effect on plant productivity⁵. Sparsely vegetated sites resulted in significantly lower evenness in bird communities²⁴. Even in the field of palaeontology, it has already been postulated that the onset of less favourable environmental conditions is indicated by lower species evenness in arthropod and sponge communities²⁵.

Biodiversity protects ecosystems against declines in their functionality and allows for adaptation to changing conditions, because the coexistence of many species provides a greater guarantee that some will back up a given function when others fail^{10,26}. Within the frame of this 'insurance hypothesis'²⁶, two aspects are important: (1) functional redundancy, in the sense of there being multiple species for each functional group^{27,28}, and (2) the relative abundances among these redundant species. At lower levels of species richness, the functionality of the ecosystem decreases⁷. In this research, all communities had the same degree of richness; hence, the importance of evenness for functional stability was isolated. Our results demonstrate that a community must have an even distribution among its functional redundant members if it is to respond rapidly to selective stress. In fact, when an ecosystem function in a highly uneven community depends strongly on the dominant species, the functional stability is endangered by environmental fluctuations⁶. Even under non-stressed conditions, high initial evenness is desirable for good functionality. Moreover, natural and anthropogenic activities influence the relative abundances more than the richness of species, and this has important consequences for ecosystems long before a species is threatened by extinction^{5,6,21}. In conclusion, the existence of a highly diverse community, where redundant species may offer equivalent contributions to a specific function, may lead to higher functional stability during environmental fluctuations⁶. This implies that changes in community evenness should warrant increased attention in biodiversity surveys.

METHODS SUMMARY

Laboratory methods. The scheme of the experimental set-up is provided in Supplementary Fig. 1. A total of 18 denitrifying species were isolated from nature (Supplementary Table 3). Denitrifiers were classified by fatty-acid methyl ester analysis and 16S ribosomal RNA gene sequencing. The different operational taxonomic units were discriminated by repetitive extragenic palindromic PCR DNA fingerprinting. By analogy with ref. 7, we considered our operational taxonomic units as 'species'. Microcosms were obtained by mixing all 18 strains in different abundances. Nitrite was added to the mixtures that were incubated

for 20 h without or with (temperature or salt) stress. The net ecosystem denitrification was estimated by the nitrite removal, which was measured spectrophotometrically (Sunrise, Tecan) as the difference of the absorbance at 540 nm before and after Montgomery reaction²⁹.

Experimental design and statistical analysis. Eighty-four different levels of initial evenness were possible, corresponding to a unique combination of Gini coefficient and X , the relative abundance of the dominant species, and each referred to as a design point (Supplementary Fig. 2). For the first experiments, each of the 84 design points was used twice. This resulted in 168 different microcosms that were placed on the multiwell plates *in duplo*. Additionally, 42 combinations of X and G were chosen according to an experimental design procedure to enable an optimal estimation of the linear and quadratic effects. The corresponding microcosms were placed *in duplo* on the multiwell plates. Model selection was performed using a series of linear models. Each of the variables and their interactions were entered sequentially and the models were compared on the basis of the AIC. The parameters of the mean model were estimated by ordinary least-squares methods. Following a residual analysis, the White estimator³⁰ was used to provide valid statistical inference in the presence of residuals with unequal variances (Supplementary Fig. 4).

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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