Diversity of habitats and bacterial communities support landscape-scale multifunctionality differently across seasons

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Abstract

In this study, we demonstrate how changes in the diversity of habitat and bacterial communities affect landscape multifunctionality. Habitat diversity may beget species diversity by increasing niche availability and resource complementarity. Species diversity, in turn, generally promotes multifunctionality, i.e. the simultaneous performance of multiple ecosystem functions. However, the relationship between habitat diversity and functioning remains to be explicitly explored. In order to test the relationship between habitat diversity and multifunctionality we constructed experimental landscapes of four different habitats common in shallow-water sediment ecosystems: cyanobacterial mats, *Ruppia maritima* meadows, silty mud and sandy beach. We manipulated the diversity of these habitats over three consecutive seasons and measured bacterial diversity, benthic microalgal diversity and four functions related to marine nitrogen cycling (gross primary production, nitrogen fixation, denitrification and uptake of dissolved inorganic nitrogen). Our results showed that higher habitat and bacterial diversity, but not benthic microalgal diversity, increased landscape multifunctionality. However, the relative importance of habitat and bacterial diversity varied with season. Habitat diversity was generally the strongest driver, affecting multifunctionality directly in summer and indirectly via bacterial diversity in autumn. In spring, neither of the two aspects of diversity was important. Our study demonstrates the importance of considering temporal differences in both habitat and species diversity for landscape multifunctionality, and the importance of direct and indirect effects in mediating ecosystem functions. Habitat homogenization in concert with loss in biodiversity can thus be a driving force of declining ecosystem functioning and the services they underpin.

Keywords: Biodiversity, microphytobenthos, structural equation modeling, benthic habitats, ecosystem functioning, marine ecosystems

Introduction

Biodiversity is the variation of life at all levels of organization, from genes to ecosystems [1]. Over the last two decades, hundreds of experiments have shown that impoverished genetic, taxonomic and functional diversity impair essential ecosystem functions such as primary production and nutrient cycling e.g. [2-5]. This fact is mainly explained by the loss of complementarity and positive interactions among organisms. The relationship between biodiversity and functioning can become stronger with increased habitat heterogeneity, e.g.

with higher spatial heterogeneity of limiting resources or higher structural diversity of the physical environment [6-9]. The proposed mechanism is that heterogeneity increases total niche space, thereby promoting the potential for species complementarity. However, the direct and indirect effects of the diversity of habitat types on the functioning within a landscape are unexplored. We suggest that habitats can facilitate each other, just as species can, and that landscape-wide functioning is promoted by habitat complementarity. One example of habitat complementarity is the interplay between mangroves and coral reefs in tropical coastal landscapes [10]. Mangroves serve as nursery habitats that affect the community structure and biomass of fish on neighboring coral reefs [11], which in turn, may protect mangrove by functioning as a breakwater [12]. Similar positive interactions may be common in landscapes that are composed of a mosaic of different habitats. Yet, many landscapes are facing biotic and abiotic homogenization due to human activities [13-15], hence the importance to understand the functional consequences of reduced habitat diversity.

While single functions can be maximized by specific species [16], a diverse assemblage of organisms is needed to maintain a suite of desired functions simultaneously, so called multifunctionality [17,18]. Analogously, diverse landscapes may be important for the concurrent provision of multiple functions. However, since habitat diversity generally favors species diversity [19], the direct and indirect effects of habitat diversity need to be separated. Moreover, the relationships between habitat diversity, species diversity and landscape multifunctionality may differ across temporal scales. Experiments that consider within-year seasonality of diversity-functioning relationships are, however, rare (e.g. Crutsinger et al. [20] and Frainer et al. [21]), as most experiments have focused on the most productive season (e.g. Reich et al. [22]). Therefore, the aim of this study was to explicitly test how changes in habitat diversity affect landscape multifunctionality–either directly or through species diversity–during different seasons.

As a model system, we used experimental landscapes consisting of four different benthic habitats common in shallow-water sediment systems: cyanobacterial mats, *Ruppia maritima* meadows, silty mud and sandy beach. Cores from each habitat were sampled in the field and arranged randomly into landscapes to form a diversity gradient including one, two, three, or four habitat types, allowing for interactions among the habitats via a common water column (Fig. S1). Shallow-water sediments are highly productive ecosystems that provide important services such as shelter and nursery grounds for secondary production. These ecosystems also serve as nutrient filters between land and sea by transforming, storing and removing nutrients from the water column [23]. To assess multifunctionality, we measured four functions related to marine nitrogen cycling mediated by microorganisms: gross primary production, nitrogen fixation, denitrification and uptake of dissolved inorganic nitrogen. We hypothesized that (i) increased habitat diversity directly enhances landscape multifunctionality through structural complementarity, (ii) habitat diversity increases bacterial and microalgal diversity, and (iii) increased bacterial and microalgal diversity increases multifunctionality. To examine if these hypotheses are valid across seasons, we repeated the experiment by sampling shallow-water sediment habitats in spring, summer and autumn. To the best of our knowledge this is the first attempt to assess the direct and indirect effects of habitat diversity on multifunctionality.

Results

Habitat, bacterial and algal diversity. Based on habitat characteristics (*see methods*), a permutational multivariate analysis of variance (PERMANOVA) showed that there was an interaction between season and habitat ($p = 0.001$). Moreover, within each season the habitats

differed during summer and autumn (p_{summer}, = 0.001, p_{autumn} = 0.002), but not in spring (p_{spring} = 0.214) (Fig. 1*A*). The bacterial community structure for all four habitats was different

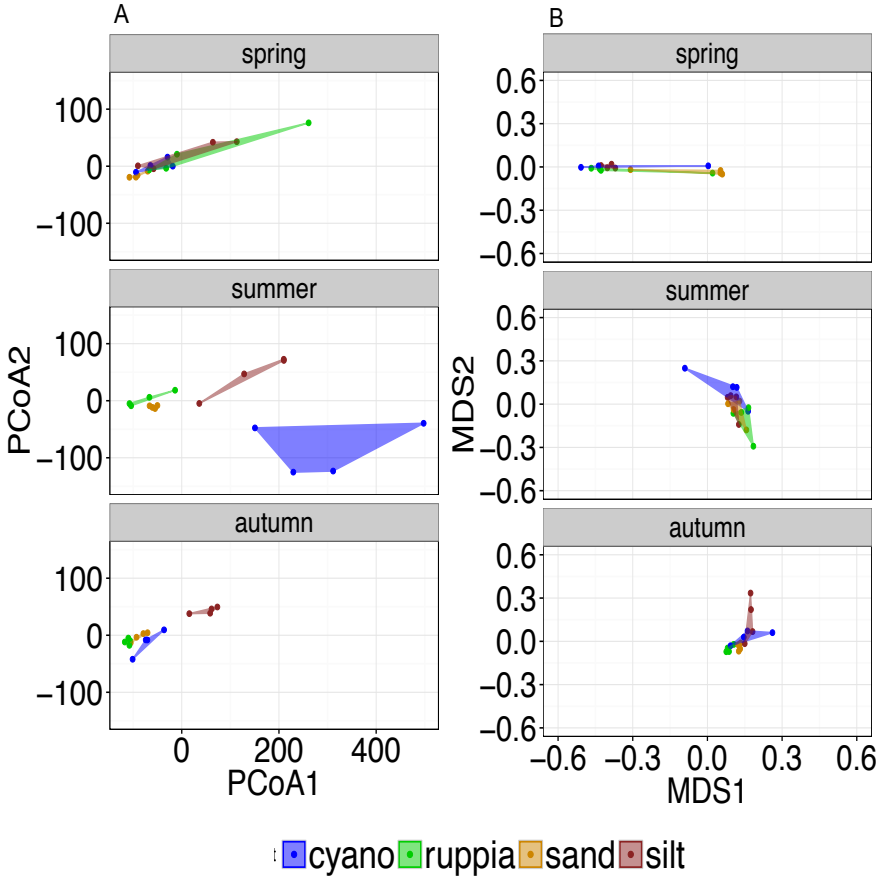

Figure 1. The within and between variability of the habitat types and the bacterial community structure during spring, summer and autumn. A) Ordination of habitat samples based on the habitat descriptors displayed in a principal coordinates analysis (PCA) plot with Euclidean distances, n = 48. B) The bacterial community structure (OTU-based) displayed in a non-metric multi-dimensional scaling (NMDS) plot based on weighted UniFrac distances, n = 48. Color codes indicate the habitat types Sandy beach (light brown), Silty mud (dark brown), Cyanobacterial mats (blue) and '*Ruppia maritima*' meadows (green).

between seasons (p = 0.006), but not between habitats within each season (p_{spring}, = 0.506, p_{summer} = 0.961, p_{autumn} = 0.261; Fig. 1*B*). The diversity of the bacterial communities (Fig. S2) increased with increasing habitat diversity during summer (p = 0.031) and autumn (p = 0.005), but not in spring (p = 0.054) (Fig. 2*A*, Table S1). Benthic microalgal diversity (Fig. S3) did not correlate with habitat diversity during any season (summer, p = 0.196; autumn, p = 0.944; spring, p = 0.340; Fig. S4, Table S2).

Temporal importance of habitat, bacterial and microalgal diversity for multifunctionality. Both habitat diversity and bacterial diversity were significantly related to landscape multifunctionality, but the effects varied with season. During spring, neither habitat diversity (p = 0.2086; Fig. 2*B*, Table S3), bacterial diversity (p = 0.7054; Fig. 2*C*, Table S4) nor algal diversity (p = 0.926; Fig. 2*D*, Table S5) affected landscape multifunctionality, which was also supported by the results from the structural equation model (SEM, Fig. 3*A*, Table S6). In summer, landscape multifunctionality increased with habitat

diversity (p = 0.002; Fig. 2*B*, Table S3), and with bacterial diversity (p = 0.022; Fig. 2*C*, Table S4), but diversity (p = 0.773; Fig. . The SEM only

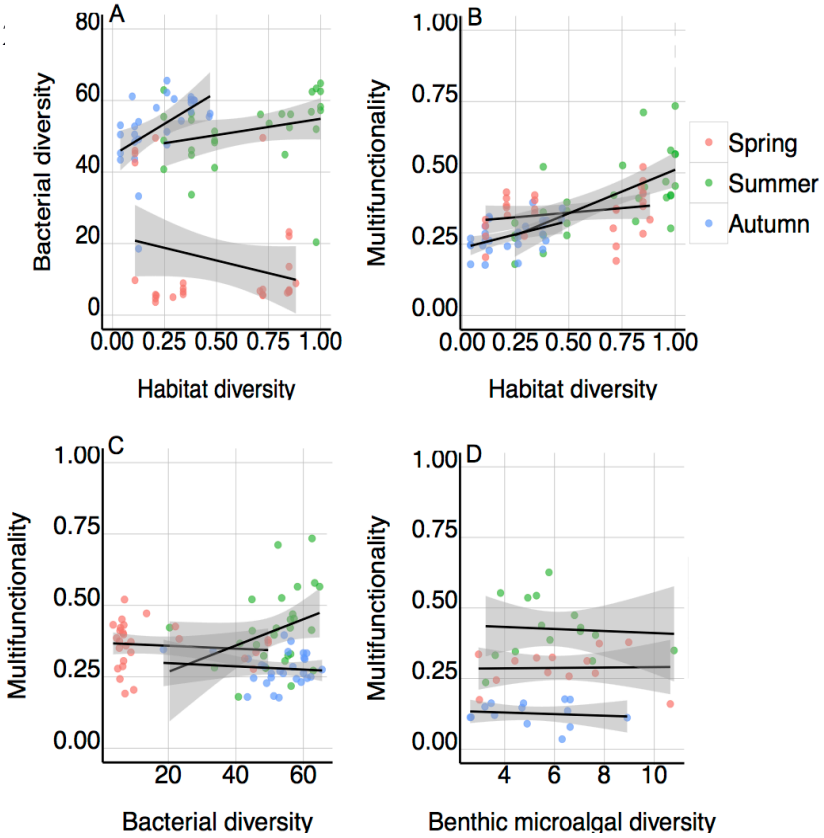

Figure 2. Linear function of relationships between habitat diversity, microbial diversity and multifunctionality across seasons. A) Relationship between habitat diversity and bacterial diversity, B) habitat diversity and index of multifunctionality (weighted average value of standardized functions), C) bacterial diversity and index of multifunctionality and D) benthic microalgal diversity (effective number of species) and index of multifunctionality. Shaded areas indicate ± 95% confidence interval, n = 84.

identified a direct effect of habitat diversity on multifunctionality ($p_{\text{habitat diversity} \rightarrow \text{multifunctionality}}$ = 0.0001), which was not meditated through increased bacterial diversity (Fig. 3*B*, Table S7). In autumn, we observed no direct effects of habitat diversity (p = 0.3038; Fig. 2*B*, Table S3), bacterial diversity (p = 0.3286; Fig. 2*C*, Table S4) or algal diversity on multifunctionality (p = 0.821; Fig. 2*D*, Table S5). However, there was an indirect effect of habitat diversity through increased bacterial diversity ($p_{\text{habitat diversity} \rightarrow \text{bacterial diversity}}$ = 0.010, $p_{\text{bacterial diversity} \rightarrow \text{multifunctionality}}$ = 0.039; Fig. 3*C*, Table S8). Among the four individual functions supporting multifunctionality, gross primary production (p = 0.008, table S9, Fig. S5*A*), nitrogen fixation ($p = 1.6\times10^{-9}$, Table S10, Fig. S5*B*) and uptake of dissolved inorganic nitrogen (p = 0.041, Table S11, Fig. S5*C*) significantly increased with habitat diversity during summer, but not in autumn or spring. Denitrification showed no trend of habitat diversity effects (Table S11, Table S12, Fig. S5*C-D*).

Net effects of habitat diversity on multifunctionality A net effect of habitat diversity was observed in summer ($F_{1,6}$ = 14.8, $p_{adj.}$ = 0.025), but not in spring or autumn ($F_{Spring1,6}$ =

1.84, $p_{adj.}$ = 0.45 and $F_{Autumn\ 1,6}$ = 1.76, $p_{adj.}$ = 0.45). In summer, mean observed multifunctionality was 0.58, and the expected multifunctionality based on the individual habitats was 0.35 (Fig. S4). Thus, the net diversity effect was 0.23.

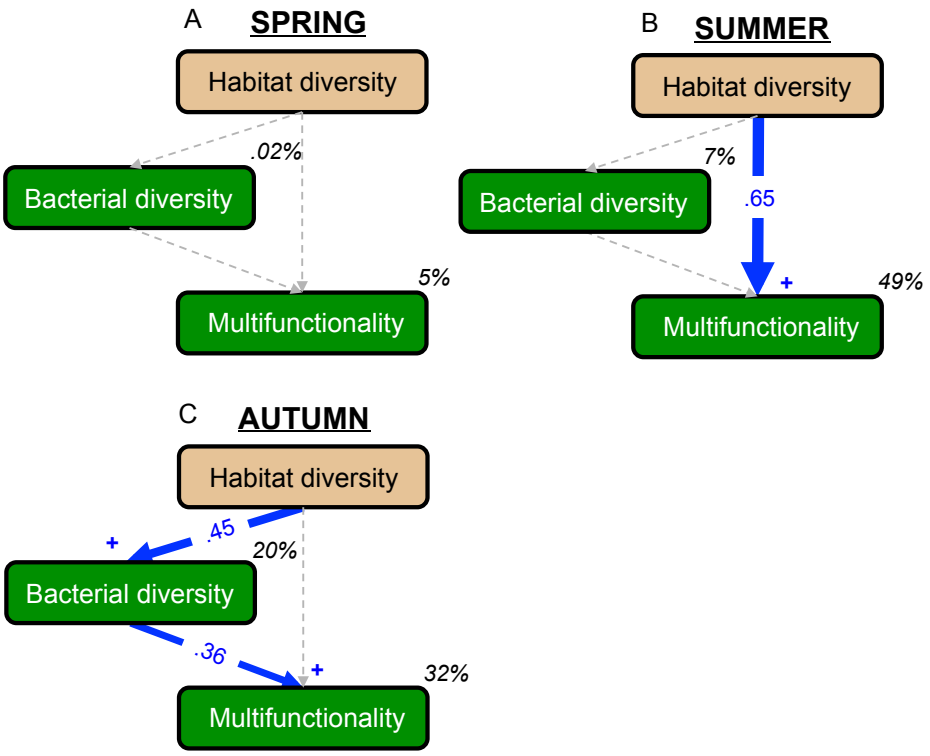

Figure 3. Path diagrams based on Structural Equation Modeling (SEM) showing how habitat and bacterial diversity affect ecosystem multifunctionality during A) spring, B) summer and C) autumn. Solid paths (blue) are statistically significant ($p < 0.05$) with standardized path coefficients indicated, whereas the dashed grey lines are not. Percentages indicate the variance explained by the model, n = 84.

Discussion

Our results show that habitat diversity is important for sustaining landscape multifunctionality in shallow-water sediments and that the effects vary by season. In summer, habitat diversity directly affected multifunctionality whereas it was mediated via bacterial diversity in autumn. During spring, neither of the two aspects of diversity was important. Hence, our study also adds to a growing body of evidence showing the importance of indirect effects in mediating ecosystem processes [24-27]. In our experiment, multifunctionality during summer was larger than the expected sum of each constituting habitat, with the highest habitat diversity showing an observed level of multifunctionality that was 67 % higher than expected (Fig. S6). This is the equivalent to overyielding at the level of species, i.e. polycultures can be more productive than monocultures due to positive interactions and niche complementarity [16]. Habitats can be complementary to each other both in terms of the species present and their physical structure [6-9]. Biogeochemical and structural characteristics of shallow-water ecosystems differ significantly, which allows for potentially complementary processes [28]. If nitrogen fixation, for example, is favored in one habitat, organismal growth may be supported in adjacent habitats with less available nitrogen. Moreover, *Ruppia* meadows affect oxygen concentration in the surrounding sediment and overlying water [29]. *Ruppia* may therefore

stimulate mineralization of organic nitrogen and further oxidation to nitrate in habitats that are typically less oxygen rich, such as silty mud.

The structure of the four habitat types in our experiment was physically, chemically and biologically different during the three seasons. Temperature and light (Fig. S7), concentrations of inorganic nutrients (Fig. S8) and organic material (Fig S9*A*-*B*) all displayed seasonal differences, which affected the habitat-defining properties. In spring, the lack of effects on multifunctionality was probably a consequence of the high within- and low between-habitat dissimilarity (Fig. 1*A*) in combination with low bacterial diversity due to the hyper-dominance of a single OTU. The structural dissimilarity of the different habitats in spring indicates physical sediment disruption causing habitat homogenization. This is a direct consequence of the winter season with erosion by freezing, ice, storms, high sedimentation rates, low light, and low availability of organic nutrients. All these environmental factors induce variation in the biogeochemical properties in sediments of the individual habitats [30]. By contrast, the four habitats were clearly separated in summer due to increased growth of autotrophic components, such as *Ruppia* meadows, cyanobacterial mats and well-developed diatom mats, higher temperature and more stable weather conditions. Thus, structural complementarity could underlie the observed direct relationship between habitat diversity and multifunctionality.

Bacterial community diversity was significantly correlated to landscape multifunctionality in summer, suggesting that taxa-based bacterial diversity *per se* was also driving the relationship. However, when bacterial diversity was analyzed simultaneously with habitat diversity the link between bacterial diversity and multifunctionality was less clear. This is in line with recent literature, showing that biogeochemistry can be the single most important driver for the functioning of bacterial dominated systems [31] and that changes in bacterial diversity often have little effect on ecosystem functioning [32] (but see Delgado-Baquerizo et al. [33]). In autumn, however, the effect of habitat diversity on landscape multifunctionality was indirect and mediated through changes in bacterial diversity. Hence, both structural complementarity and bacterial diversity are linked to landscape multifunctionality, but the relative importance can be context dependent since habitat biogeochemistry, primary productivity and bacterial diversity vary with season [30, 34]. Changes in ecosystem components can thus affect the relative importance of habitat and bacterial diversity for landscape multifunctionality.

Benthic microalgal diversity was unrelated to landscape multifunctionality during all three seasons. Since it was unfeasible to identify all benthic microalgal taxa to species, the resolution might have been insufficient to detect potential differences in diversity. A general positive effect of species richness of primary producer on production has previously been shown [5, 35], but the consequences of changes in benthic microalgal diversity are not well understood. An observational study relating benthic microalgal diversity to functioning found both positive and negative relationships [36]. The only study so far in which benthic microalgal richness was manipulated reported positive effects of diversity on production [37]. The maximum richness, however, was only eight species, and natural benthic microalgal communities are far more diverse [36].

The present study has demonstrated a direct link between habitat diversity and landscape-scale multifunctionality, an observation that was partly mediated by effects associated to bacterial diversity. None of the examined relationships were constant across seasons, which stresses the importance of considering temporal effects. Diversity and community composition of other types of microbiota, such as meiofauna and protists, might also play a role for ecosystem multifunctionality. Yet, a full mechanistic elucidation of all relationships between the biotic and abiotic components of habitat diversity on landscape-scale multifunctionality was beyond the scope of our study and warrants further research. An

aspect of habitat diversity not considered in our study is the dispersal and migration of organisms between the habitats. Examples of such aspects are the observed large-scale synergistic effects between coral reefs and mangrove ecosystems [11] and the small-scale dispersal that affects the biodiversity–functioning relationship [38] within metacommunities. From a management perspective, our results suggest that habitat homogenization can have negative consequences for landscape functioning and the ecosystem services they underpin.

Material and Methods

Model ecosystem and experimental set-up. Natural, intact sediment habitats of 'Sandy beach', 'Silty mud', 'Cyanobacterial mats' and '*Ruppia maritima* meadows were sampled on the west coast of Sweden during the summer and autumn of 2013 and spring 2014 using hollow plastic cylinders (ID = 8 cm, h = 11cm). In total, 112 habitat cores were sampled per season (Sandy = 29, Silty = 29, Cyano = 26, Ruppia = 28), and randomly assembled into 4 diversity levels (1 – 4), by placing 4 habitat cores in one larger cylinder (ID = 25 cm, h = 25 cm), representing 1 replicate. For diversity level 1, one landscape consisted of 4 cores from the same habitat type and each landscape was replicated 4 times (4 x 4 = 16 landscapes in total). The landscapes with habitat diversity levels 2-4 consisted of a mixture of different habitat types: 2+2 for diversity 2, 1+1+2 for diversity 3 and 1+1+1+1 for diversity 4. The cylinders containing the experimental landscapes were placed in a greenhouse with a continuous water flow of surface water pumped from an adjacent bay (Fig. 1S). Each experiment was run for 2 weeks.

Habitat descriptors. Porosity was calculated as the percentage weight loss following drying of ~ 30 g wet sediment to constant weight (24 h). The density of wet sediment was measured on 20 ml sediment. The organic carbon and nitrogen content was analyzed in ~ 30 mg dried sediment through vapor phase acidification using a Carlo Erba NA 1500 elemental analyzer. To extract sediment pore water, 50 mL of sediment was centrifuged (32 000 G) for 30 min (Eppendorf Centrifuge 5810 R), the pore water was filtered (0.45 μm surfactant-free cellulose acetate filters, MinisartH) and immediately frozen –80°C. *Ruppia maritima* was rinsed, dried and weighed (g. dry weight m^{-2}). Microalgal pigments (chlorophyll *b*, echinenone, fucoxanthin) were analyzed using high performance liquid chromatography according to the method of Wright and Jeffrey (1987) [39] (*all environmental data are available in* Table S13 – S15).

Habitat diversity. The 10 habitat descriptor variables were z-transformed, and multivariate Euclidian distances were calculated for all pairwise habitat combinations and among the replicates within each habitat. The habitat diversity of a given landscape was defined as the sum of all pairwise distances between the constituting habitats. The resulting values for habitat diversity were scaled between 0 and 1 by dividing all values (across habitat diversity levels and seasons) by the maximum total Euclidian distance, which was the summer experiment with diversity level 4. Differences in habitat diversity among landscapes were assessed statistically using multivariate homogeneity of groups' dispersion (function 'betadisper' within the 'vegan' package in R) [40]. To determine whether there were significant differences among the habitat types, we used permutational multivariate analysis of variance implemented in the 'adonis' function within the 'vegan' package in R [40]. The clustering of the samples was displayed with Euclidian distances in a PCA plot (Fig. 1*A*).

Landscape functions. To calculate gross primary production, light and dark fluxes of oxygen were measured with UniSense oxygen optodes to estimate net primary production and

community respiration, respectively. Dissolved inorganic nitrogen (DIN) concentrations were assessed by measuring ammonium and nitrate + nitrite according to standard colorimetric procedures [41]. For potential denitrification, 2 ml of homogenized sediment from all replicates of each habitat type was added to 10 ml gas-tight exetainers flushed with helium and pre-incubated for 24 hours at in-situ temperature to remove oxygen and nitrate present in the sediment. Eighty mL stock solution of $^{15}NO_3$ ($Na^{15}NO_3$, 99.6 atom %, Europa Scientific Ltd) was added to each exetainer, resulting in a concentration of 50 µM in the sediment pore water. Samples were shaken and incubated in the dark for 2.5 hours. The incubation was terminated by addition of 6.1 mol L^{-1} $ZnCl_2$. Sediment samples were analyzed at the stable isotope laboratory at UC Davis.

Rates of nitrogen fixation were measured using the Acetylene Reduction Assay [42] modified according to Capone (1993) [43]. Sediment from each habitat type was homogenized and four subsamples (3 mL each) were added to four 5.8 mL cylindrical glass Exetainers® fitted with gas-tight rubber septa (Labco Limited). In each Exetainer, 1.5 mL of filtered seawater water was added, leaving approximately 1.3 mL of untreated air as headspace. The headspace was then enriched with ~ 20% acetylene (C_2H_2) gas (v:v). Incubations were terminated after 0, 24, 48, and 72 h by addition of 0.1 mL of 6.1 mol L^{-1} $ZnCl_2$. Nitrogen fixation was assessed by extracting 250 μL of headspace and analyzing the concentration of ethylene using a gas chromatograph (Hewlett Packard 5890, series 1). The ethylene production was recalculated to atmospheric N fixation per square meter and day using a ratio of 1 N_2 fixed per 3 ethylene molecules produced [43, 44].

Bacterial community diversity metric and structure. From the cores in the experiments, one sediment sample (~ 5 g) was taken for each unique habitat, such that the number of samples corresponded to the habitat diversity level (1-4). For sediment cores of the same habitat within one landscape, the samples were pooled. The sediment samples were stored at -20 °C. For each sample, DNA was extracted from 0.3 g of sediment using the FastDNA® Spin Kit for Soil (MP Biomedical) according to the manufacturer's instructions. The amount of extracted DNA was quantified using the Qubit® fluorometer and reagents (Life Technologies Corporation). Bacterial community structure and diversity was assessed by amplification and sequencing of the V3-V4 region within the 16S rRNA gene using a two-step protocol [45] and the universal primer pro341F and pro805R [46] with Nextera adaptor sequences (Illumina, Inc.). A total of 33 cycles were used (25 + 8) and the annealing temperature in both PCR cycles was 55°C. Sequencing was performed by Microsynth (Microsynth AG, Switzerland) on the MiSeq platform (Illumina) using 2 x 300 bp paired-end chemistry.

The resulting sequences were trimmed using FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit), and paired-end sequences were merged using PEAR [47] with a minimum overlap of 30 bases, quality score threshold of 30 for the two consecutive bases, and the minimum length of 300 bp for the assembled sequences. Quality filtering was performed using USEARCH v8.0 (Drive5, [48]) using a maximum expected error ("maxee") value of 1. The final set of sequences was then assigned to OTUs at the 97% sequence similarity level using the USEARCH61 algorithm [49] within QIIME v1.8.0 [50]. We aligned the representative sequences of all OTUs in QIIME, using the pynast algorithm and the pre-aligned greengene database (v.13_8) as template [51-53]. The phylogenetic tree was built with FastTree and midpoint rooting and made ultrametric using the program PATHd8 [54].

We calculated bacterial diversity for each experimental landscape as effective phylogenetic diversity of order q = 1, following the method developed by Marcon and Hérault 2015 [55] based on Chao et al., 2010 [56] (Fig. S8). The metric corresponds to the number of

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species in an equally diverse community where all species have the same abundance and are equally related to each other. To calculate landscape-wide bacterial diversity, the reads of the single habitats within each landscape were first rarefied and subsequently summed. At habitat diversity levels 3, where one habitat was present twice, the weighted sum was calculated. We excluded three landscapes, where ≥50% the samples were missing, but kept two landscapes where only a single habitat was missing (two replicates of habitat diversity level 4 in summer). For these two samples, bacterial diversity was calculated by replacing the reads of the missing habitats by the average read numbers of the corresponding habitats from the other replicates (with the same habitat diversity level and season). Bacterial community dissimilarity was calculated as abundance weighted UniFrac distance between the communities, based on OTU data. All data manipulation was performed in R [57] and bacterial diversity was calculated with the entropart package [58].

Benthic microalgal diversity metrics. From the cores in the experiments, sediment samples were taken from the top 5 mm sediment using a 2-ml cut-off syringe. Live cells (with fluorescing chlorophyll) were counted in a Bürker counting chamber using epifluorescence microscopy at 500 × magnification. Cells were identified to the nearest taxon level possible, measured and allocated to size groups. We calculated diversity as effective number of taxonomical units of order q=1 [59] (Fig. S9).

Calculations of multifunctionality and statistics. Multifunctionality was calculated as the weighted average of the standardized function values [60]. All functions were first recalculated and standardized to be positive by adding the lowest value to all data, and then standardized by the maximum observed value. The weighted average index was calculated by taking the average of all functions and subtracting the standard deviation [61]. The relationship between habitat diversity, bacterial diversity, microalgal diversity and the weighted average index (as well as all individual functions) was fitted with a linear model with multifunctionality as the dependent variable, season as a categorical independent variable, habitat diversity as an independent continues variable, and the interaction between season and habitat diversity.

We also compared the observed multifunctionality in the communities consisting of four habitat types with what we would expect based on the multifunctionality in each single habitat types. This is what is commonly referred to as the net diversity effect (Div_{net}) [16]. It is defined as the difference between the observed an the expected effect: $Div_{net} = Div_{obs} - Div_{exp}$), where Div_{obs} is the observed multifunctionality at the focal diversity level (in our case the community with all four habitat types) and Div_{exp} is the expected multifunctionality in the focal diversity treatment based on the single habitat communities.

Structural Equation Modelling (SEM). To test the relative contribution of habitat and species diversity on ecosystem multifunctionality we analysed our data within a SEM framework. We only used bacterial diversity since we did not have sufficient amount of data of benthic microalgal diversity to run a SEM model. First, data were separated into three groups – spring, summer and autumn – and analysed with a multigroup SEM [62], since there were seasonal differences in the effects of habitat diversity on bacterial diversity and multifunctionality. Secondly, we performed a comparative fit evaluation between models with or without a direct path between habitat diversity and multifunctionality. The difference in AIC (59.978 for the model with no direct path to MF and 54.000 for the model with a direct path to MF) indicated that the fully mediated model with a direct path to multifunctionality was the best model for further analysis. Since we have little doubt about the causal structure in the model, the evaluation of our SEM model is a strictly confirmatory analysis, meaning

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that data are compared to only a single model and no model statistics (chi-square, df and p-value) are available since the model is fully saturated. Significance levels for individual paths between variables were set at $\alpha = 0.05$. Structural equation models were run in AMOS (version 20).

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References

1. CBD (2005) *Handbook of the Convention on Biological Diversity (Including its Cartagena Protocol on Biosafety)*. 3 ed (Montreal, Canada, Earthscan Publications Ltd)
2. Hughes AR, et al. Ecological consequences of genetic diversity. (2008) *Ecol Lett* 2008 11(6):609-623.
3. Cardinale BJ, et al. The functional role of producer diversity in ecosystems. (2011) *Am J Bot* 98(3):572-592.
4. Cardinale BJ, et al. Biodiversity loss and its impact on humanity. (2012) *Nature* 486(7401):59-67.
5. Gamfeldt L, et al. Marine biodiversity and ecosystem functioning: what's known and what's next? (2015) *Oikos* 124(3):252-265.
6. Tylianakis JM, et al. Resource heterogeneity moderates the biodiversity-function relationship in real world ecosystems. (2008) *PLoS Biol* 6(5):947-956.
7. Cardinale BJ, Ives AR, Inchausti P, Effects of species diversity on the primary productivity of ecosystems: extending our spatial and temporal scales of inference. (2004) *Oikos* 104(3):437-450.
8. Griffin JN, et al. Spatial heterogeneity increases the importance of species richness for an ecosystem process. (2009) *Oikos* 118(9):1335-1342.
9. Angelini C, et al. Foundation species' overlap enhances biodiversity and multifunctionality from the patch to landscape scale in southeastern United States salt marshes. (2015) *Philos Trans R Soc Lond B Biol Sci* 282:20150421
10. Harborne AR, et al. The functional value of Caribbean coral reef, seagrass and mangrove habitats to ecosystem processes (2006) *Advances in Marine Biology,* eds Southward AJ, Young CM, Fuiman LA, (Academic press, Vol 50) pp.57-189.
11. Mumby PJ, et al. Mangroves enhance the biomass of coral reef fish communities in the Caribbean. (2004) *Nature* 427(6974):533-536.
12. Ferrario F, et al. The effectiveness of coral reefs for coastal hazard risk reduction and adaptation. (2014) *Nat Commun* 5(3794).
13. McKinney ML and Lockwood JL, Biotic homogenization: a few winners replacing many losers in the next mass extinction. (1999) *Trends Ecol Evol* 14(11):450-453.
14. Thrush SF, et al. Predicting the effects of habitat homogenization on marine biodiversity. (2006) *Ecol Appl* 6(5):1636-1642.
15. Hewitt J, et al. A latent threat to biodiversity: consequences of small-scale heterogeneity loss. (2010) *Biodivers Conserv* 19(5):1315-1323.
16. Loreau M, and Hector A, Partitioning selection and complementarity in biodiversity experiments. (2001) *Nature* 413(6855):548-548.

17. Duffy JE, Richardson JP, Canuel EA. Grazer diversity effects on ecosystem functioning in seagrass beds. (2003) *Ecol Lett* 6(7):637-645.
18. Lefcheck, J.S., et al., Biodiversity enhances ecosystem multifunctionality across trophic levels and habitats. (2015) *Nat Commun* 6(6936).
19. Stein A, Gerstner K, Kreft H. Environmental heterogeneity as a universal driver of species richness across taxa, biomes and spatial scales. (2014) *Ecol Lett* 17(7):866-880.
20. Crutsinger GM, et al. Temporal dynamics in non-additive responses of arthropods to host-plant genotypic diversity. (2008) *Oikos* 117(2):255-264.
21. Frainer A, McKie BG, Malmqvist B. When does diversity matter? Species functional diversity and ecosystem functioning across habitats and seasons in a field experiment. (2014) *J Anim Ecol* 83(2):460-469.
22. Reich PB, et al. Impacts of Biodiversity Loss Escalate Through Time as Redundancy Fades. (2012) Science 336(6081):589-592.
23. McGlathery KJ, Sundbäck K, Anderson IC Eutrophication in shallow coastal bays and lagoons: the role of plants in the coastal filter. (2007) *Mar Ecol Prog Ser* 348:1-18.
24. Grace JB, et al. Causal networks clarify productivity-richness interrelations, bivariate plots do not. (2014) *Func Ecol* 28(4):787-798.
25. Alsterberg C, et al. Consumers mediate the effects of experimental ocean acidification and warming on primary producers. (2013) *Proc Natl Acad Sci USA* 110(21):8603-8608.
26. Jones CM, et al. Recently identified microbial guild mediates soil N2O sink capacity. *Nature Clim Change* 4(9):801-805.
27. Wootton JT Indirect effects, prey susceptibility, and habitat selection – impacts of birds on limpets and algae. (1992) *Ecology* 73(3):981-991.
28. Eyre BD, et al. Metabolism of different benthic habitats and their contribution to the carbon budget of a shallow oligotrophic sub-tropical coastal system (southern Moreton Bay, Australia). (2011) *Biogeochemistry* 102(1-3):87-110.
29. Thursby GB Root-exuded oxygen in the aquatic angiosperm Ruppia Maritima. (1984) *Mar Ecol Prog Ser* 16(3):303-305.
30. Sundbäck K, Miles A, Göransson E Nitrogen fluxes, denitrification and the role of microphytobenthos in microtidal shallow-water sediments: an annual study. (2000) *Mar Ecol Prog Ser* 200:59-76.
31. Powell JR, et al. Deterministic processes vary during community assembly for ecologically dissimilar taxa. (2015) *Nat Commun* 6(8444).
32. Roger F, et al. Multiple dimensions of bacterial diversity unrelated to functioning, stability and multifunctionality. (2016) *PeerJ PrePrints* 4(1688-1681).
33. Delgado-Baquerizo M, et al. Microbial diversity drives multifunctionality in terrestrial ecosystems. (2016) *Nat Commun* 7(10541)
34. Bolhuis H, Stal LJ Analysis of bacterial and archaeal diversity in coastal microbial mats using massive parallel 16S rRNA gene tag sequencing. (2011) *Isme J* 5(11):1701-1712.
35. Cardinale BJ Biodiversity improves water quality through niche partitioning. (2011) *Nature* 472(7341):86-113.
36. Vanelslander B, et al. Complementarity effects drive positive diversity effects on biomass production in experimental benthic diatom biofilms. (2009) *J Ecol* 2009. 97(5):1075-1082.
37. Forster RM, et al. Biodiversity-ecosystem function relationship in microphytobenthic diatoms of the Westerschelde estuary. (2006) *Mar Ecol Prog Ser* 311:191-201.

38. Gonzales A, Mouquet N, Loreau M Biodiversity as spatial insurance: the effects of habitat fragmentation and dispersal on ecosystem functioning (2009) *Biodiversity, Ecosystem Functioning and Human Wellbeing: An ecological and economical perspective,* eds Naeem S, Bunker DE, Hector A, Loreau M, Perrings C (Oxford University Press, Oxford). pp. 134-146.
39. Wright SW, Jeffrey SW Fucozanthin pigment markers of marine-phytoplanktion analyzed by HPLC and HPTLC. (1987) *Mar Ecol Prog Ser* 38(3):259-266.
40. Oksanen J, et al. *vegan: Community Ecology Package. R package version 2.2-1.* (2015) http://CRAN.R-project.org/package=vegan
41. Strickland JD, Parsons TR A practical handbook of seawater analysis (1972) Fisheries Research Board of Canada, *2nd ed* (The Alger Press)
42. Stewart WDP, Fitzgera GP, Burris RH In situ studies on N2fixation using acetylene reduction technique. (1967) *Proc Natl Acad Sci USA* 58(5):2071-2078.
43. Capone DG Determination of nitrogenase activity in aquatic samples using the acetylene reduction procedure (1993) *Handbook of methods in aquatic microbial ecology,* eds Kemp PF, Sherr BF, Sherr EB, Cole JJ (CRC Press LLC) pp. 621-631.
44. Capone DG, et al. Nitrogen fixation by Trichodesmium spp.: An important source of new nitrogen to the tropical and subtropical North Atlantic Ocean. (2005) Glob Biogeochem Cycles 19(2).
45. Berry D, et al. Barcoded Primers Used in Multiplex Amplicon Pyrosequencing Bias Amplification. (2012) *Appl Environ Microbiol* 78(2):612-612.
46. Takahashi S, et al. Development of a Prokaryotic Universal Primer for Simultaneous Analysis of Bacteria and Archaea Using Next-Generation Sequencing. (2014) *Plos One* 9(8).
47. Zhang J, et al. PEAR: a fast and accurate Illumina Paired-End reAd mergeR. (2014) *Bioinformatics* 30(5):614-620.
48. Edgar RC, Flyvbjerg H Error filtering, pair assembly and error correction for next-generation sequencing reads. (2015) *Bioinformatics* 31(21):3476-3482.
49. Edgar RC Search and clustering orders of magnitude faster than BLAST. (2010) *Bioinformatics* 26(19):2460-2461.
50. Caporaso JG, et al. QIIME allows analysis of high-throughput community sequencing data. (2010) *Nature Methods* 7(5):335-336.
51. Price MN, Dehal PS, Arkin AP FastTree: Computing Large Minimum Evolution Trees with Profiles instead of a Distance Matrix. (2009) *Mol Biol Evol* 26(7):1641-1650.
52. Britton T, et al. Estimating Divergence Times in Large Phylogenetic Trees. (2007) *Syst Biol* 56(5):741–752.
53. Caporaso JG, et al. PyNAST: a flexible tool for aligning sequences to a template alignment. (2010) *Bioinformatics* 26(2): p. 266-267.
54. Britton T, et al. Estimating divergence times in large phylogenetic trees. (2007) *Syst Biol* 56(5):741-752.
55. Marcon E, Herault B Decomposing phylodiversity. (2015) *Methods Ecol Evol* 6(3):333-339.
56. Chao A, Chiu CH, Jost L Phylogenetic diversity measures based on Hill numbers. (2010) *Philos Trans R Soc Lond B Biol Sci* 365(1558):3599-3609.
57. Development Core Team R (2010) R: A Language and Environment for Statistical Computing (R Foundation for Statistical Computing, Vienna).
58. Marcon E, Hérault B An R Package to Measure and Partition Diversity. (2015) *J Stat Softw* 67(1):1-26.
59. Jost L Entropy and diversity.(2006) *Oikos* 113(2):363-375.

60. Pasari JR, et al. Several scales of biodiversity affect ecosystem multifunctionality. (2013) *Proc Natl Acad Sci USA* 110(25):10219-10222.
61. Pasari JR, Levi T, Zavaleta ES, Tilman D (2013) Several scales of biodiversity affect ecosystem multifunctionality *Proc Natl Acad Sci USA* 110(25)10219-10222
62. Grace JB, Jutila H The relationship between species density and community biomass in grazed and ungrazed coastal meadows (1999). *Oikos* 85(3):398-408.