

## ORIGINAL ARTICLE

# Individual-level trait diversity predicts phytoplankton community properties better than species richness or evenness

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**Understanding how microbial diversity influences ecosystem properties is of paramount importance. Cellular traits—which determine responses to the abiotic and biotic environment—may help us rigorously link them. However, our capacity to measure traits in natural communities has thus far been limited. Here we compared the predictive power of trait richness (trait space coverage), evenness (regularity in trait distribution) and divergence (prevalence of extreme phenotypes) derived from individual-based measurements with two species-level metrics (taxonomic richness and evenness) when modelling the productivity of natural phytoplankton communities. Using phytoplankton data obtained from 28 lakes sampled at different spatial and temporal scales, we found that the diversity in individual-level morphophysiological traits strongly improved our ability to predict community resource-use and biomass yield. Trait evenness—the regularity in distribution of individual cells/colonies within the trait space—was the strongest predictor, exhibiting a robust negative relationship across scales. Our study suggests that quantifying individual microbial phenotypes in trait space may help us understand how to link physiology to ecosystem-scale processes. Elucidating the mechanisms scaling individual-level trait variation to microbial community dynamics could there improve our ability to forecast changes in ecosystem properties across environmental gradients.**

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## Introduction

Functional traits can help illuminate the relationship between biodiversity and ecosystem processes (e.g. Norberg *et al.*, 2001; Norberg, 2004; Hillebrand and Matthiessen, 2009; Reiss *et al.*, 2009). Most research in this area has thus far largely focused on taxonomic or phylogenetic richness as measures of biodiversity (see reviews by Cardinale *et al.*, 2011; Krause *et al.*, 2014). However, the importance of relative species densities for ecological processes has been neglected (Mulder *et al.*, 2004; Hillebrand *et al.*, 2008; Zhang *et al.*, 2012), as well as the functional redundancy or diversity of organisms (including intraspecific variation) that may partially decouple the number of

species from the functions supported by a community (Chase and Leibold, 2003).

Traits, which are often analysed in terms of species mean values, may be better defined as the features of *individual* organisms' phenotypes that determine fitness and life history (McGill *et al.*, 2006; Violle *et al.*, 2007). They influence ecological interactions and dynamics (e.g. Bolnick *et al.*, 2011; de Bello *et al.*, 2011; Violle *et al.*, 2012) and have important consequences for population demography and ecosystem processes (Norberg, 2004; McGill *et al.*, 2006; Cadotte *et al.*, 2011). Empirical evidence for the importance of individual trait variation for ecosystem properties is still lacking, despite the fact that intraspecific trait variance contributes significantly to plant community functional responses to environmental change (Siefert and Ritchie, 2016; Volf *et al.*, 2016). The importance of intraspecific variation for explaining community properties has mostly been explored theoretically in ecology (Bolnick *et al.*, 2011; Albert *et al.*, 2012; Violle *et al.*, 2012; De Laender *et al.*, 2014; Barabás and

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D'Andrea, 2016; Hart *et al.*, 2016). We expect that, for example, changes in trait diversity (TD) should reflect natural selection mechanisms, which affect processes at all scales of community and ecosystem organisation (Matthews *et al.*, 2011). Additionally, competition for resources, predation, environmental change and rates of mutation and plasticity will all affect individual phenotypes and consequently TD within and between taxa, which will in turn influence population demography, community dynamics and ecosystem properties (Norberg *et al.*, 2001). The link between individual-level TD and large-scale processes is underexplored in real communities, particularly in microorganisms (Gsell *et al.*, 2013; Ackermann, 2015; Schreiber *et al.*, 2016).

Individual-level traits in microbial assemblages have become easier to measure owing to recent technological developments (Shade *et al.*, 2009; Pomati *et al.*, 2011; Fontana *et al.*, 2014; Krause *et al.*, 2014). Indices that quantify community TD using individual-level data (thereby integrating inter- and intraspecific trait variation) have also been recently tested and developed (Fontana *et al.*, 2016). Hereafter, we refer to them as 'individual-level TD indices' because, despite quantifying community properties, they are calculated using traits measured on individual organisms. These indices jointly describe the three independent components of TD (richness, evenness and divergence, respectively): the trait onion peeling (TOP; Fontana *et al.*, 2016) index is the sum of all successive convex hulls' areas touching the individuals of a community in multidimensional trait space, the trait even distribution (TED; Fontana *et al.*, 2016) index is the regularity in the distribution of individuals when compared with a perfectly even reference distribution, and the functional dispersion index (FDis; Laliberté and Legendre, 2010) is the mean distance of individuals to the centroid of trait distribution. The TOP index has been successfully applied to high-throughput individual-level data to study the role of phenotypic variation over time in the adaptation of microbial populations to environmental perturbations (Krismer *et al.*, 2017). It reflects changes in the trait space coverage, which may happen as a consequence of environmental filtering

both within and at the edges of trait distribution. TED is related to the reciprocal distances between neighbour individuals in the trait space and is likely to reflect biotic interactions (Fontana *et al.*, 2016). FDis distinguishes communities where individuals are closer to the centroid of the multidimensional trait distribution from communities where extreme phenotypes are found (Fontana *et al.*, 2016).

In this study, we focus on natural phytoplankton communities, whose diversity and dynamics are tightly linked to large-scale biogeochemical processes. These microorganisms form the basis of aquatic food webs and are responsible for almost 50% of total global primary production (Field *et al.*, 1998). The TD metrics described above have allowed us to explore the relative strength of individual-level trait variation and taxonomic diversity metrics (species richness and evenness), as predictors of important ecosystem properties—phytoplankton community biomass and resource-use efficiency.

We studied these ecosystem properties in 28 lakes from two European regions (Switzerland and Danube Delta, Romania), sampled at different temporal and spatial scales (Supplementary Table S1). We related these properties to the TD and taxonomic diversity metrics, while accounting for variation in water chemistry and physics. This heterogeneity across sites allowed us to sample environmental responses in terms of individual-level TD change or turnover of species, and their effects on community properties. We used microscopy for classification of algal species and scanning flow cytometry (SFC) to estimate total biovolume (hereafter biomass) and morphological and physiological traits measured on individual cells/colonies (Table 1, more details in Supplementary Table S2). We tested all combinations of species richness, evenness, TOP, TED and FDis using linear mixed-effects model averaging to quantify and compare their predictive ability on biomass and resource-use efficiency, while controlling for environmental variables.

We expected that the inclusion of individual-level TD would improve the variance explained by statistical models predicting phytoplankton productivity. In our SFC data, we cannot distinguish between inter- and intraspecific trait variation because we are unable to classify cells into species

**Table 1** Ecological relevance of the measured phytoplankton traits

| Measure   | Ecological importance                                  |
|---|--|
| Length of the particle                                      | Reproduction, resource acquisition, predator avoidance |
| Frontal shape of the particle                               | Resource acquisition                                   |
| Fluorescence of chlorophyll <i>a</i>                        | Resource acquisition                                   |
| Fluorescence of phycoerythrin <sup>a</sup>                  | Resource acquisition                                   |
| Fluorescence of accessory and decaying pigments             | Resource acquisition                                   |
| Evenness in the distribution of pigments within cell/colony | Resource acquisition                                   |
| Cell rugosity/internal structure/gas vesicles/thylacoids    | Resource acquisition, predator avoidance               |

List of the seven traits used for calculating trait diversity indices (Litchman and Klausmeier, 2008; Pomati *et al.*, 2013).

<sup>a</sup>For Lake Zurich data (only one laser), this measure is missing.

groups. Therefore, our assessment of the importance of intraspecific trait variation is only indirect: if interspecific trait variation is more important in influencing ecosystem properties, models including TD indices (i.e. trait richness) should not improve on those that incorporate species-level metrics (i.e. species richness), which are expected to be good proxies of TD in this case. However, if intraspecific trait variation has an important role, standard biodiversity metrics will be poorer predictors of ecosystem properties than TD indices.

## Materials and methods

### *Phytoplankton communities at three study sites*

To test relationships between biodiversity indices and ecosystem properties, we used monitoring data from three different lake systems, covering a wide range of temporal and spatial scales. Lake Greifensee, Lake Zurich and Danube Delta (with 26 lakes), in this order, represent a gradient of increasing sampling time span (3 months, 7 months, 2 years), decreasing sampling frequency (weekly, monthly, seasonally) and increasing spatial scale (6 depths from 1 to 8.5 m, 11 depths from 0 to 40 m, 26 lakes in a geographical region). All data sets include microscopy data (phytoplankton classification, enumeration and biomass calculation) obtained using the Utermöhl method (Utermöhl, 1931), as well as phytoplankton morphological and physiological traits acquired by SFC, in addition to physical and chemical water parameters. Supplementary Table S1 summarises the characteristics of the three lake systems.

In lake Greifensee (Switzerland), monitoring data were collected from a single location at the North end of the lake by an automated system that integrates physical, chemical (Idronaut, Brugherio, Italy; <http://www.idronaut.it>) and biological analyses (Pomati *et al.*, 2011). From the 31st of July to the 24th of October 2014, SFC measurements were collected every 4 h from samples collected at six different depths (1, 2.5, 4, 5.5, 7 and 8.5 m). Additional chemical analyses and microscopy measurements (to obtain species diversity metrics) were performed on water samples collected manually at the same location, depths and time (average time interval between successive samples = 58.3 h, standard deviation = 67.7 h). Total phosphorus (TP) and total nitrogen (TN) were measured using DIN Standards (German Institute for Standardisation). The sample size is 207 (36 time points x 6 depths, with some missing data points due to technical problems).

In lake Zurich (Switzerland), monitoring data were collected from a single location in the centre of the lake by the Zurich drinking water company (WVZ), which also performed microscopy and chemical analyses as described in Pomati *et al.* (2012). Water was sampled at 11 different depths (0, 1, 2.5, 5, 7.5,

10, 12.5, 15, 20, 30 and 40 m) from the 6th of May to the 2nd of December 2009, with a time interval of 1 month. The same water samples were analysed with SFC. The sample size is 82 (8 months x 11 depths, with some missing data points due to technical problems).

Monitoring data were collected from 26 shallow lakes of the Danube Delta (Romania). These lakes form a complex system in a region of hundreds of km<sup>2</sup> (Supplementary Table S1). From a single location in each lake, an integrated sample over the water column was collected in spring, summer and autumn of two following years (May, July and September 2013; May, July and October 2014). The same water samples were used for microscopy and chemical analyses, as well as SFC measurements. TP and TN were measured using standard colorimetric methods (Tartari and Mosello, 1997). The sample size is 136 (6 months x 26 lakes, with some missing data points due to technical problems).

### *SFC measurements*

The scanning flow cytometer Cytobuoy (Woerden, The Netherlands; <http://www.cytobuoy.com>) was used for counting and characterising phytoplankton single cells and colonies (e.g. Dubelaar *et al.*, 2004; Pomati *et al.*, 2013; Fontana *et al.*, 2014). The present instrument contains two laser beams (coherent solid-state sapphire, wavelengths 488 and 635 nm), but the samples from lake Zurich were measured with a previous version of the instrument containing only one laser (wavelength 488 nm). Light, both from forward scattering (FWS) and sideward scattering, provides information on particle morphology. The fluorescence emitted by photosynthetic pigments was measured by three different detectors referred to as red (668–734 nm range; FL.Red from the 488 nm laser and FL.2.Red from the 635 nm laser), orange (601–668 nm range; FL.Orange) and yellow (536–601 nm range; FL.Yellow). Cytobuoy measurements provide time-resolved pulse signals, from which many descriptors of morphology, internal structure and fluorescence profile of each particle were extracted. More details on the instrument can be found elsewhere (Dubelaar *et al.*, 2004; Pomati *et al.*, 2011, 2013; Fontana *et al.*, 2014).

### *Individual-level TD indices*

For all phytoplankton samples, we calculated the TD indices describing the three components of TD defined in Mason *et al.* (2005): richness, evenness and divergence. We quantified these three TD components using distinct indices: TOP, TED and FDis, respectively (Laliberté and Legendre, 2010; Fontana *et al.*, 2016). These TD indices fulfil theoretical requirements that make them suitable for application to individual-based measurements, when every organism constitutes a unique combination of traits and therefore influences the TD

(Fontana *et al.*, 2014). Thus, in the present study we did not identify neither taxonomic nor functional groups in the SFC data, and we calculated TD metrics without classifying particles (single cells represent the fundamental unit in this study). These multivariate indices were calculated using seven Cyto buoy-derived traits, selected on the basis of their ecological and physiological relevance for phytoplankton (Table 1), and to avoid trait multicollinearity: length (maximum value between length by sideward scattering and length by FWS), average FWS, average FL.Red, average FL2.Red, average FL.Yellow, fill factor FL.Red, ratio between average sideward scattering and average FWS. Owing to this *a priori* selection of traits, there was no need for dimensionality reduction. In all data sets the absolute value of pairwise Pearson's correlation coefficients between the selected traits were below 0.7, which we chose as the maximal acceptable limit to reduce collinearity issues (Dormann *et al.*, 2013). Before calculating TD indices, the selected traits were standardised (mean = 0; standard deviation = 1) so that each trait has equal weight (Petchey and Gaston, 2006). This was necessary because the selected traits have different units and vary in value by orders of magnitude. Details about the interpretation of the selected traits and their ecological relevance are reported in Supplementary Table S2. These seven traits provide information on phytoplankton three-dimensional structure, fluorescence properties, cell/colony size and distribution of pigments and other structures within cells (Pomati *et al.*, 2013). Although they do not cover all relevant dimensions of trait space in phytoplankton (e.g. life history, nutrient uptake kinetics and mixotrophy), these traits relate to photosynthesis, resource acquisition (surface-to-volume ratio), reproduction and predator avoidance (Litchman and Klausmeier, 2008; Pomati *et al.*, 2013; Table 1).

#### *Species-level biodiversity metrics*

We calculated species richness (number of species) and Pielou's evenness using microscopic counts of the phytoplankton community in each sample. Effort was made to standardise the counting method and taxonomic identification (at the species level) across all data sets. The same sample preparation method was used (Utermöhl, 1931) and taxonomists exchanged knowledge and information. Pielou's evenness was calculated based on the biovolume of each species present, derived by multiplying the abundance data from microscopy by the best available estimate of species-specific biovolume.

#### *Biomass calculation*

Biomass was defined as the sum of the biovolumes of all phytoplankton cells or colonies in the samples, divided by the sample volume analysed. The biovolume of each single particle was estimated

assuming an ellipsoid shape with the formula:  $(\text{Biovolume}^2 = 0.0017 \times \text{FWS} \cdot \text{Total} - 0.013)$  (Foladori *et al.*, 2008; Pomati and Nizzetto, 2013). It is important to note that total FWS was derived by SFC, but it is not one of the traits included in the calculation of the TD indices (see above).

#### *Model selection and averaging*

We formulated linear mixed-effects models to explain variation in ecosystem properties. We included all possible combinations of five explanatory variables as fixed effects: species richness, Pielou's evenness and the three metrics of individual-level TD (TOP, TED and FDis). TD estimates are not independent from taxonomy-based biodiversity measures. However, we found these two groups of predictors to be only weakly correlated (a maximum Pearson's  $r$  of 0.50 across all data sets, between TED and Pielou's evenness in lake Zurich). In addition, we accounted for temporal and spatial autocorrelation in abiotic (physics and chemistry) and biotic environmental variables (e.g. grazers and parasites). First, we included the date of sampling as a fixed effect, with a quadratic term. In the case of lake Greifensee (high temporal sampling frequency), the exact time of sampling and the date were combined in a single variable. Second, random intercepts were also included in all models to account for spatial ecosystem heterogeneity: depth of sampling for lakes Greifensee and Zurich, and lake identity for the shallow Danube Delta lakes. This approach resulted in a list of 32 models for each data set. The variance inflation factors of all variables of interest were lower than 4 in all models, indicating that multicollinearity was not a problem.

We calculated marginal  $R^2$  values (Nakagawa and Schielzeth, 2013) to estimate the proportion of variance explained by the fixed effects alone in each model. Models with delta correction to the Akaike information criterion  $< 7$  were defined as the strongest set of models (Burnham and Anderson, 2002; Richards, 2005). These were subsequently used for model averaging to minimise the dependence of the regression coefficients on single models and to assess overall predictive power. Model selection and averaging were performed using the *MuMIn* R-package (R Core Team, 2013).

#### *Additional analyses*

We also performed all the above analyses using resource use efficiency (RUE, *sensu* Ptacnik *et al.*, 2008) as ecosystem property instead of biomass, which allowed us to study the predictive power of biodiversity metrics on the ability of a community to take up limiting resources. We defined RUE as the amount of standing phytoplankton biomass per unit of limiting resource present (Ptacnik *et al.*, 2008). We used phytoplankton total biovolume (calculated with SFC data) as a proxy for biomass and TP as the main

limiting resource in temperate lakes (Ptacnik *et al.*, 2008), which in turn is a proxy for potential system productivity. Therefore, RUE was calculated as (biomass/TP).

Additionally, we ran a full set of analyses including in all models the number of particles measured by SFC as a fixed effect, to account for variation in biomass or RUE caused by SFC sample size.

To examine the influence of phytoplankton limiting resources on TED, we used high-resolution data from lake Greifensee including photosynthetic active radiation (PAR, from high-frequency profiles) and TP (representing potential availability), the key limiting factors for algal growth in freshwater environments (Ptacnik *et al.*, 2008). We performed a multiple linear regression analysis ( $N=195$ ) with PAR, TP (both log-transformed) and their interaction as explanatory variables, and TED as response variable.

## Results

The best models retained for model averaging explained more than 60% of the variance in phytoplankton biomass, with a large proportion contributed by the biodiversity metrics (Figure 1, Table 2 and Supplementary Tables S3–S5). Temporal structure in the data contributed variable amounts depending on the lake system (Table 2). Note that marginal  $R^2$  values represent the variance explained by the fixed effects alone, and therefore do not include the variance attributable to spatial autocorrelation (random factor). Models containing individual-level TD metrics improved predictions of microbial biomass and RUE. Our results show a consistent negative correlation between TED and community biomass across data sets. The other components of TD (richness and divergence), after correcting for the number of cells/colonies, had a nonsignificant effect on ecosystem properties (Supplementary Figures S2 and S3, and Supplementary Tables S10–S17), except TOP in lake Greifensee where it had, however, a weaker effect than TED (Supplementary Figures S2a and S3a).

Species richness was not a significant predictor of biomass. A positive relationship in the Danube Delta lakes disappeared when the models accounted for the number of individuals measured by SFC (Supplementary Figure S2c and Supplementary Tables S10 and S13), suggesting a sampling effect.

The different lake systems showed some idiosyncratic patterns, but the main results were consistent. In lake Greifensee, TED was the strongest predictor of biomass, while TOP was the second (Table 2 and Figure 1a). Species richness, Pielou's evenness and FDis were nonsignificant predictors of biomass (Figure 1a). In Lake Zurich, Pielou's evenness was the strongest predictor of biomass, while TED was the second (Table 2 and Figure 1b). Species richness, TOP and FDis were nonsignificant predictors of biomass (Figure 1b). In the Danube Delta lakes, TOP

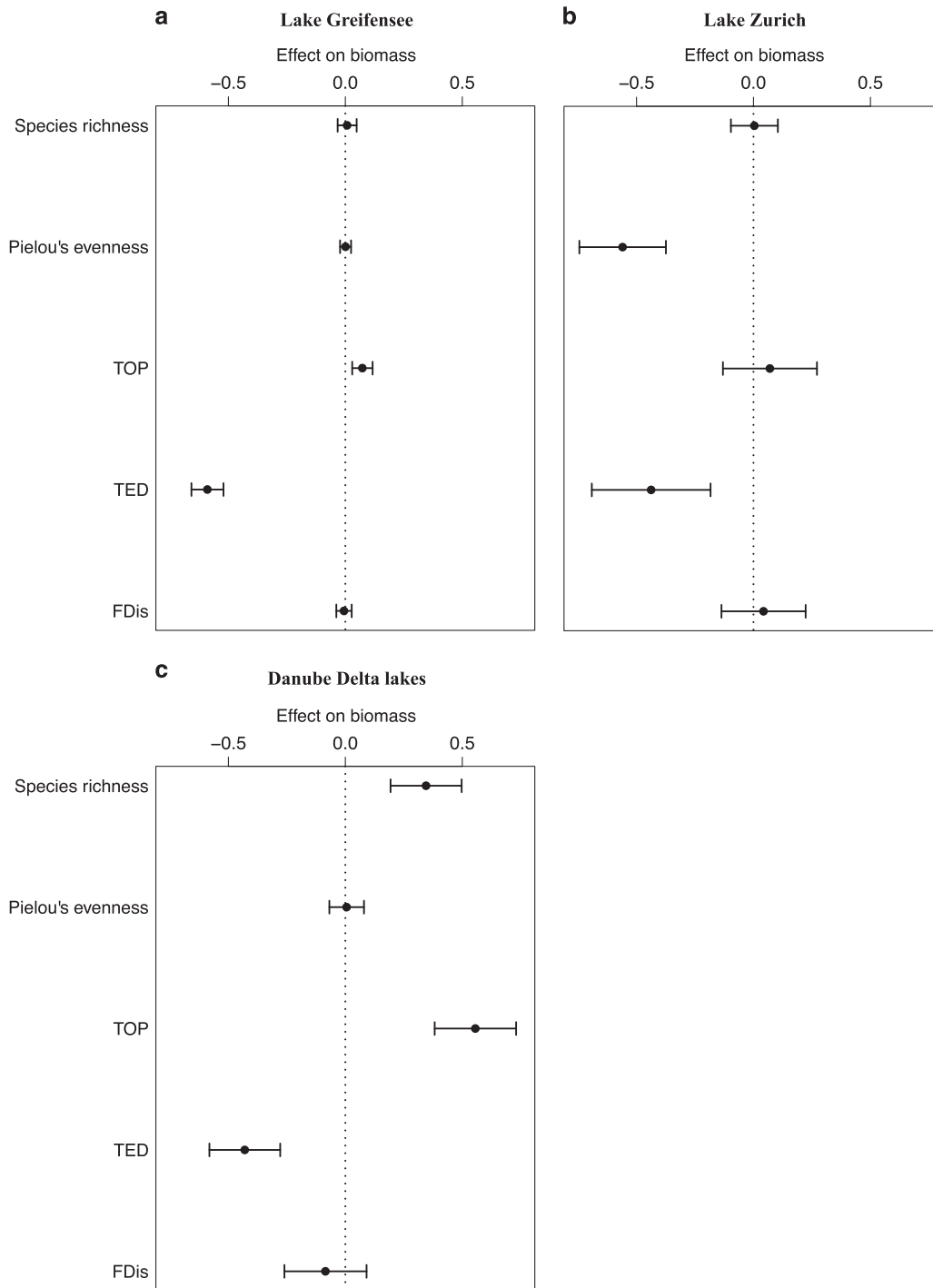
was the strongest predictor of biomass, whereas TED and species richness were the second and third, respectively (Table 2 and Figure 1c). Pielou's evenness and FDis were nonsignificant predictors of biomass (Figure 1c).

Identical analyses using RUE instead of biomass as the ecosystem property of interest yielded almost identical patterns as those presented in Figure 1 (Supplementary Figure S1 and Supplementary Table S6). Including in all models the number of individuals measured by SFC as a fixed effect, to correct for potential biases associated with the fact that some biodiversity metrics, especially TOP (Fontana *et al.*, 2016), are increasing functions of sampled abundance, also did not change the results (Supplementary Figures S2 and S3 and Supplementary Tables S10–S17). These additional steps had the effect of reducing the predictive strength of species richness and TOP, when analysing both biomass and RUE in the Danube Delta lakes (Supplementary Figures S2c and S3c). TED was, however, the strongest predictor of ecosystem properties (Supplementary Figures S2 and S3) under all analytical conditions.

A multiple linear regression to test the influence of the main limiting resources on TED revealed a negative, significant effect of PAR and TP ( $P=0.03$  and  $P<0.001$ , respectively;  $R^2=0.25$ ). TED was highest under limitation by both light and nutrients, with a marginal effect ( $P=0.07$ ) of their interaction (Figure 2).

## Discussion

This study demonstrates that individual-level trait metrics may help link biodiversity in natural microbial communities to essential ecosystem properties. In particular, we found that TED (regularity in the distribution of individual phenotypes in trait space) was the most important predictor of community productivity. Previous studies have explored the correlation between TD measures and ecosystem properties in macro- and microorganisms, but trait evenness has rarely emerged as being important (Tilman *et al.*, 1997; Mouillot *et al.*, 2011; Bílá *et al.*, 2014). Gagic *et al.* (2015) found contrasting effects of trait evenness, whereas Santos *et al.* (2014) showed a negative correlation between trait evenness and phytoplankton productivity, consistent with our results. These differences are probably due to the fact that the tested relationships are dependent on environmental heterogeneity (Norberg *et al.*, 2001; Hodapp *et al.*, 2016). It has to be noted, however, that the concept of trait evenness used in previous studies is radically different from the one used here, where regularities in trait distances among individuals represent the unit of measure. Previous studies calculated TD using species-level data, disregarding the individual-level trait differences that we find to be the most important predictors.



**Figure 1** Effect sizes of biodiversity metrics on biomass. TED is the strongest predictor of biomass among the five biodiversity metrics. Values are standardised model-averaged regression coefficients with 95% confidence intervals, derived from models accounting for temporal and spatial heterogeneity.

Individual-level trait evenness, as characterised by TED, is also conceptually different from species evenness, as, by measuring the distances of individual organisms in multidimensional trait space, it does not include any information about relative abundances (Supplementary Figure S4; Fontana *et al.*, 2016). To understand the negative correlation between TED and community biomass in our data, it

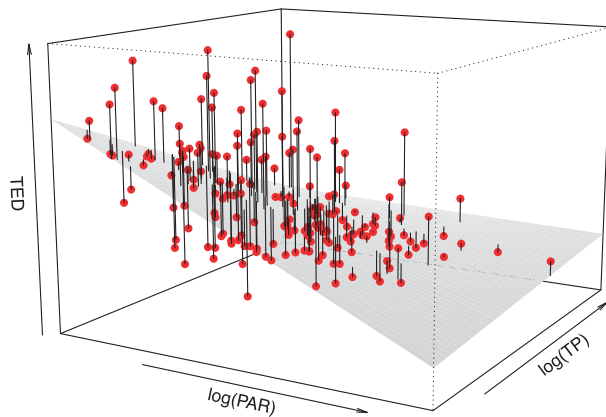
is necessary to refer to environmental effects on both, although they cannot be fully disentangled given the correlative nature of our analyses. Our interpretation of the negative relationship between TED and total biomass is that resource scarcity indirectly affected both in opposite ways—decreasing total community biomass and increasing TED (Figure 2). The pattern that we observed may be explained by plasticity or

**Table 2** Summary of the results with biomass as response variable

|   | <i>Greifensee</i> (N = 207)    | <i>Lake Zurich</i> (N = 82)    | <i>Danube delta lakes</i> (N = 136) |
|---|--------------------------------|--------------------------------|-------------------------------------|
| Number of models used for model averaging (out of 32) | 8                              | 8                              | 4                                   |
| Sum of weights  | 98.8%                          | 99.5%                          | > 99.9%                             |
| Species richness estimate (95% CI)                    | 0.008 (−0.033, 0.048)          | 0.004 (−0.097, 0.104)          | <b>0.345</b> (0.193, 0.497)         |
| Pielou's evenness estimate (95% CI)                   | 0.001 (−0.023, 0.024)          | <b>−0.559</b> (−0.744, −0.374) | 0.006 (−0.068, 0.080)               |
| TOP estimate (95% CI)                                 | <b>0.073</b> (0.029, 0.117)    | 0.070 (−0.131, 0.271)          | <b>0.556</b> (0.382, 0.730)         |
| TED estimate (95% CI)                                 | <b>−0.590</b> (−0.658, −0.521) | <b>−0.437</b> (−0.691, −0.184) | <b>−0.430</b> (−0.581, −0.278)      |
| FDis estimate (95% CI)                                | −0.006 (−0.039, 0.027)         | 0.043 (−0.137, 0.223)          | −0.085 (−0.260, 0.091)              |
| Mean $R^2$ (null model <sup>a</sup> )                 | 0.65 (0.02)                    | 0.63 (0.24)                    | 0.65 (0.17)                         |

Abbreviations: CI, confidence interval; FDis, functional dispersion index; TED, trait even distribution; TOP, trait onion peeling. The estimates of the five explanatory variables (with 95% CIs) represent standardised model-averaged regression coefficients. Values in bold are significant at the  $P < 0.05$  level.

<sup>a</sup>Accounting for temporal and spatial heterogeneity.



**Figure 2** Relationship between phytoplankton limiting resources and TED. Individual-level trait evenness (TED) increases with decrease in PAR and TP in lake Greifensee ( $N = 195$ ). The grey surface represents the fitted linear model relating TED to  $\log(\text{PAR})$ ,  $\log(\text{TP})$  and their interaction.

selection for particular phenotypic characters under conditions of resource limitation. This may induce individual microbes to be more evenly spaced in a multidimensional landscape defined by their functional traits (thereby minimising the overlap of ‘individual niches’). Our analyses confirmed that low levels of both light and nutrients maximised TED values in microbial communities (Figure 2).

We believe that resource limitation constrained the total productivity of the phytoplankton communities in our study, inducing individual phenotypes to spread more evenly along trait axes (to better exploit available resources), leading to the negative correlation between TED and community biomass. This interpretation does not contradict the expected outcomes of competition under resource limitation, which leads to the dominance (at the population level) of the species with the highest affinity for resources (Tilman, 1982). Variation in TED signals individual organisms converging around certain trait combinations (low TED) or spreading more regularly among trait space (Supplementary Figure S4); this happens as a consequence of co-occurring physiological and ecological processes. While both

acclimation and competitive dominance could explain the above patterns, the fact that individual-level TD dominates the predictive power of our statistical models relative to species-based metrics (considered here as proxies of trait change due to taxa turnover) suggests that variation in TED may be explainable mainly by physiological responses at the single-cell level or genetic variation within species.

Light limitation can induce changes in the distribution of individual phytoplankton fluorescence traits because both the absolute and relative intracellular abundance of different pigments can be regulated, thereby changing absorbance across the light spectrum. This has been previously shown in the cyanobacterium *Tolypothrix tenuis*, in which algal cells regulated the relative amounts of different pigments to fill gaps in the prevailing light spectrum that were not already exploited by competitors (Stomp *et al.*, 2004). And in plant communities, intraspecific trait variability is influenced by light acquisition traits of neighbour individuals (Le Bagousse-Pinguet *et al.*, 2015). These studies showed that a complementary effect caused by the differential use of light is not always a consequence of increased taxa richness (as in Striebel *et al.*, 2009a, b), but is in principle possible at the intraspecific level. Light represents a spectrum of wavelengths, being a potentially infinite set of resources that can be exploited by photosynthetic organisms able to regulate pigment composition (Stomp *et al.*, 2007a, b, 2008).

The relationship between TP and TED is more difficult to interpret as many phytoplankton traits might be involved in optimising phosphorus uptake rate and use (Litchman and Klausmeier, 2008; Table 1). In this context, cell size represents a key trait, which reflects different nutrient acquisition strategies by determining surface-to-volume ratio (Litchman and Klausmeier, 2008). The fact that patterns observed for RUE are very similar to those of biomass (Supplementary Figure S1 and Supplementary Tables S6–S9) suggests, however, that the TP–TED relationship might be less important than the PAR–TED relationship. If the relationship between ecosystem properties and TED was

driven by limiting TP, then it would have disappeared when biomass was corrected for TP (i.e. considering RUE), which was not the case.

In our data, species richness, which represents the most commonly used metric in studies correlating biodiversity with ecosystem properties (Cardinale *et al.*, 2011), did not show a strong relationship with biomass. Interestingly, species richness showed no clear correlation with TD metrics across our data sets (maximum absolute value of Pearson's  $r$  was 0.40). Our results seem to contradict previous studies (e.g. Hector *et al.*, 1999; Cardinale *et al.*, 2011) and the common assumption of a positive correlation between species richness and primary production. However, nonsignificant as well as negative correlations have been already reported previously (Cardinale *et al.*, 2011). One possible reason for the lack of importance of species richness in our study is that we focused on natural communities shaped by natural selection gradients, whereas studies correlating biodiversity with ecosystem properties typically deal with communities assembled randomly from a species pool (Hooper *et al.*, 2005; Krause *et al.*, 2014). Natural communities are the result of assembly processes over abiotic and biotic gradients and their species composition reflects the adaptation to a specific environment. In this context, phenotypic composition might have a more important role compared with the number of species present. However, Ptacnik *et al.* (2008) found a significant positive relationship between genus richness and RUE in natural algal communities. These authors investigated a very large spatial (almost continental) and temporal scale (over several years), whereas our largest scale is regional (Danube Delta) with a generally shorter temporal frequency. This suggests that the scale of analysis might also be important when considering the relative importance of biodiversity metrics in explaining ecosystem processes (Farnsworth, 1998; Cadotte *et al.*, 2011; Chalmandrier *et al.*, 2017).

The importance of species evenness for ecosystem properties has been intensively tested in the past few years using experimental, observational and modelling approaches, with partially contradictory results (positive effect of evenness through niche partitioning vs dominance of high productive species). Most studies have focussed on grassland and forest communities (e.g. Mulder *et al.*, 2004; Zhang *et al.*, 2012; Orwin *et al.*, 2014; Dooley *et al.*, 2015), but some microbial communities have also been investigated (Filstrup *et al.*, 2014; Powell *et al.*, 2015). In this study, species evenness emerged as important only in lake Zurich (Figure 1), where it was the strongest predictor of biomass. This result likely reflects the specific characteristics of this lake's phytoplankton community: in the past few decades, the ecosystem has been dominated by a single cyanobacterial species (*Planktothrix rubescens*) that accounts for ~40% of the total annual phytoplankton biomass (Posch *et al.*, 2012). Variation in species

evenness is principally driven by growth of this cyanobacterium, which causes fluctuations in community biomass (blooms). This is consistent with patterns observed in many other lakes (Filstrup *et al.*, 2014).

Ecosystem processes depend upon guilds of interacting organisms and their aggregated responses to their immediate environment. Theoretically, individual-level trait variation is important to maintain ecosystem processes only in a spatially or temporally heterogeneous environment (under constant environmental conditions the fittest phenotype would eventually prevail) (Norberg *et al.*, 2001; Norberg, 2004; Hodapp *et al.*, 2016). The importance of individual-level TD in our analyses suggests that (1) there is significant environmental heterogeneity in the studied aquatic ecosystems, which may lead to the emergence and maintenance of TD (Ackermann, 2015; Stark *et al.*, 2017), and (2) this heterogeneity (characterised by our TD indices) is important to understand and predict ecosystem processes. As TD can characterise phenotypic variation among individual organisms, aggregating physiological (plastic) responses of phenotypes, evolution of populations and ecological turnover of species, it has been long hypothesised to perform better than species-level metrics in explaining ecosystem properties (Norberg *et al.*, 2001; Petchey and Gaston, 2006; Fontana *et al.*, 2014, 2016). Despite variation between systems, including TD metrics in statistical or mechanistic models explaining phytoplankton productivity may allow us to improve our predictive power over a range of environmental gradients. The relationship between trait evenness and both environment and ecosystem properties deserves further investigation, both theoretical and empirical. Experimental work can help disentangle the mechanisms (physiology/evolution) that determine variation in the regularity of algal phenotypes in trait space, its dependency on environmental conditions and its importance for ecological interactions.

#### Data accessibility

The data sets analysed during the current study are available in the Zenodo repository at <https://doi.org/10.5281/zenodo.848273>.

#### Conflict of Interest

The authors declare no conflict of interest.

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