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Microbial species performance responses to environmental changes: genomic traits and nutrient availability

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Abstract. How microbial species performance indicators, such as growth rate and carbon assimilation rate, respond to environmental changes is a challenging question, especially for complex communities. This limits our ability to understand how species performance responses to environmental changes (that is, species environmental responses) of microbes could be linked to genomic traits and nutrient availability. Based on stable isotope labeling of DNA, we propose a new approach with effect-size metrics to quantify the species environmental responses of microbes by comparing the species performance between defined control and treatment groups. The species performance within microbial communities of the natural or altered environments could be quantitatively determined with quantitative stable isotope probing (qSIP). We further apply this approach, namely effect-size qSIP, to measure species environmental responses upon carbon and nitrogen additions for soil bacteria on mountainsides and to understand their responses from the perspective of genomic traits. Towards high elevations, there is a stronger nitrogen limitation that is indicated by the higher aggregated responses, measured as community-weighted means, of bacterial growth rate upon nitrogen additions. The aggregated responses are further explained by genomic traits, which show higher percentages of significant Kyoto Encyclopedia of Genes and Genomes (KEGG) orthologues (KOs) and more diverse KEGG pathways under nutrient additions including nitrogen, and further improve the explanatory power of microbial environmental responses. Nitrogen-induced responses at the species level show the strongest associations with essential KOs for rare species, whereas carbon-induced responses show the strongest associations for dominant species. We conclude that, in addition to environmental determinants such as nitrogen limitation, genomic traits are extremely important for predicting microbial environmental responses at both the community and species levels. Taking advantage of this new approach at the species level, we reveal that rare and dominant species differentially respond to nutrient enrichment via their metabolic traits. The approach and findings can lead to a more holistic understanding of microbial environmental responses in natural habitats, which will be essential for predicting microbial community responses to global environmental changes.

Key words: carbon assimilation rate; genomic traits; growth rate; nutrient availability; quantitative stable isotope probing; species environmental responses; species performance.

Introduction

How does microbial species performance respond to environmental changes? Such a simple question is challenging to answer with quantitative measurements, especially for complex communities. This question remains, partially because of a lack of pure culture isolates for most microbial species, which hinders the determination of their physiology and consequently their functional roles. Overcoming this challenge, however, is becoming feasible with culture-independent techniques such as quantitative stable isotope probing (qSIP), which can quantitatively measure species performance within natural communities based on stable isotope labeling of DNA (Hungate et al. 2015). More recently, microbial species performance such as soil bacterial growth and carbon assimilation rates, quantified by qSIP, have been shown to be constrained more by evolutionary history than by contemporary environments, even along a broad climatic gradient (Morrissey et al. 2019). Further, microbial traits could be inferred from genomes or metagenomes (referred to as "genomic traits") (Barberan et al. 2014, Fierer et al. 2014, Hartman et al. 2017, Malik et al. 2020) and affect the performance of an organism under environmental changes (Violle et al. 2007, Lennon

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et al. 2012). For example, genome size, rRNA operon copy numbers and orthologous gene content can predict bacterial growth rates in natural habitats, such as soils (Li et al. 2019), and changes in bacterial species in response to resource perturbations in pure cultures (Treseder et al. 2011, Roller et al. 2016, Ho et al. 2017). At the whole-community level, microbial performance can be influenced by environmental conditions such as organic matter source and nutrient enrichment (Taylor and Townsend 2010, Mooshammer et al. 2014, Hartman et al. 2017). Community-aggregated performance across phylogenetically diverse species has the potential to predict the ecosystem-level implications of microbial processes (Fierer et al. 2014). Nevertheless, the constraints imposed by environmental determinants and genomic traits on the responses of microbial performance to environmental changes are rarely assessed at the species level in complex communities such as from natural habitats.

Here, we proposed a new approach named effect-size qSIP (Box 1; Fig. 1) to evaluate the effect sizes of responses of bacterial species performance to environmental changes (that is, species environmental response) by comparing the species performance between defined control and treatment groups. For instance, the species performance such as the growth rate and carbon assimilation rate could be measured with qSIP, and we then quantified the magnitude and direction (i.e., positive or negative) of species performance responses to environmental alterations such as nutrient resource changes, soil carbon, and nitrogen availabilities with effect-size metrics. Furthermore, we evaluated the validity and generality of the proposed approach at both species and community levels by re-examining the unprecedented qSIP data of 96 soil samples reported in Morrissey et al. (2019) to recalculate bacterial species performance in four ecosystems along the C. Hart Merriam elevational gradient of 1,760–2,620 m. These ecosystems include desert grassland, pinyon–juniper woodland, ponderosa pine forest, and mixed conifer forest (Morrissey et al. 2019). In addition to demonstrating this new approach to natural microbial communities, we also aimed to examine (1) the elevational patterns of aggregated species performance and environmental responses, measured as community-weighted means (CWM); (2) the drivers, such as environmental determinants for the microbial environmental responses at the community level; (3) the influences of genomic traits, including genomic signatures and Kyoto Encyclopedia of Genes and Genomes (KEGG) orthologues (KOs), on microbial environmental responses at both the community and species levels; and (4) the divergent effects of KOs on species environmental responses between rare and dominant species (Fig. 1). We showed that this new approach could be easily applied to quantify the responses of microbial species performance in natural environments upon environmental changes. We also revealed that environmental determinants, such as nitrogen limitation, and genomic traits are extremely important for microbial environmental responses at both the community and species levels, and that rare and dominant species differentially respond to nutrient enrichment via their metabolic traits.

Box 1. Glossary

Species performance: Microbial species functions, such as growth rate and carbon assimilation rate, which could be driven by contemporary environments and species traits.

Species environmental response: A change in microbial species performance upon external environmental alteration, such as nutrient enrichment and temperature variation. Such species environmental response could be upscaled to a community-by-community level-aggregated measure such as community-weighted mean. Microbial environmental responses could thus be quantified at both species and community levels.

Genomic traits: Genomic features of microbial species such as genome structure (e.g., genome size, guanine-cytosine content, rRNA operon number, and the number of genes) and the abundance of functional genes or proteins, which could be inferred from genomes or metagenomes.

qSIP: Quantitative stable isotope probing, which quantitatively measures species performance within natural habitats.

Effect size qSIP: A qSIP-based quantitative measure of microbial species responses to environmental alteration, which can be measured with effect-size metrics, such as Hedges' g.

Community-weighted mean (CWM): A community level-aggregated measure of a species performance, species environmental response, or trait, which is based on community composition (that is, species relative abundances) and reflects the mean species behavior or feature.

Materials and Methods

Experimental design

We reanalyzed previously reported qSIP data (Morrissey et al. 2019). More details on sample collection, incubation conditions and molecular analyses are described in Morrissey et al. (2019). Briefly, soils were collected in October 2014 from four ecosystems along the C. Hart Merriam elevational gradient near Flagstaff, Arizona, USA. Within each ecosystem, triplicate soil samples were collected for stable isotope incubations. The incubations involved exposing 1.0 g of dry soil with a 70% water-holding capacity to a glucose solution of 1,000 μg C/g soil or a nitrogen solution of 100 μg N/g soil in the

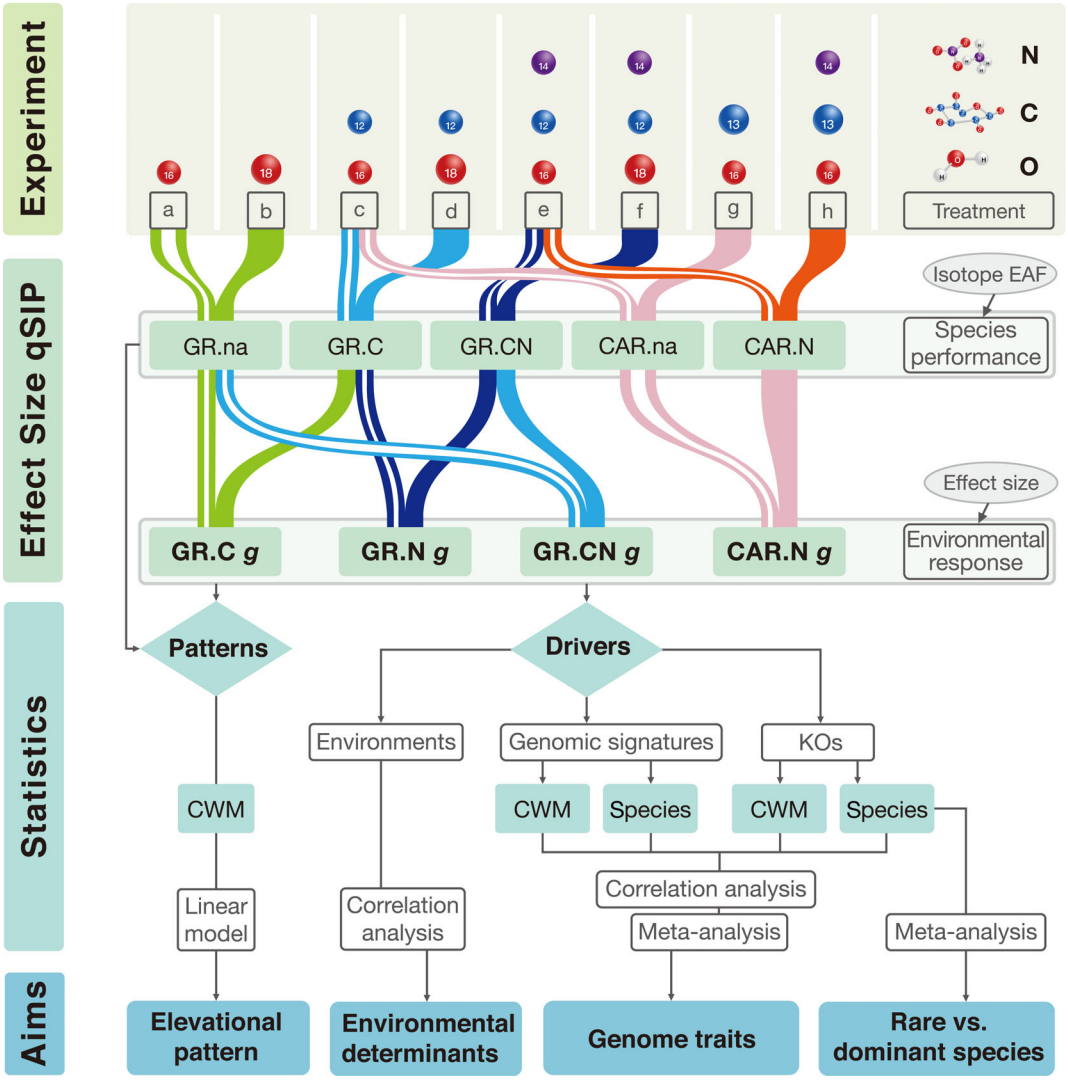

FIG. 1. The framework outlining our experimental setups, methods, and aims. For experimental setups, there were eight isotope–substrate treatments (that is, (a)–(h)) with the combination of NH_4NO_3, glucose and water additions. Using quantitative stable isotope probing (qSIP), we measured the excess atom fraction (EAF) of ^{18}O for the growth rate (GR) or ^{13}C for the carbon assimilation rate (CAR) under five conditions of species performances. For GR, the three conditions were: without added substrates (GR.na), with added carbon (GR.C) and with added carbon and nitrogen (GR.CN). For CAR, there were two conditions: with added carbon (CAR.na) and with added carbon and nitrogen (CAR.N). These species performances in GR and CAR were the same as reported in Morrissey et al. (2019). However, we further proposed a novel approach named effect-size qSIP, which quantifies the effect sizes of responses of microbial species performance to nutrient additions (that is, species environmental responses) by comparing the defined control and treatment groups. There were four metrics of species environmental responses assessed by Hedges' *g*: GR under the addition of carbon (GR.C *g*), nitrogen (GR.N *g*), and carbon and nitrogen (GR.CN *g*), and CAR under nitrogen addition (CAR.N *g*). For each metric, we defined treatment and control groups (indicated by hollow and solid color lines, respectively) relatively, according to what nutrient effects were to be examined. For instance, when considering the effect of nitrogen on GR, the treatment and control groups are GR.CN and GR.C, respectively. Finally, we aimed to examine (1) the elevational patterns of aggregated species performance and environmental responses, measured as community-weighted means (CWM); (2) the environmental determinants on microbial environmental responses at the community level; and (3) the influences of genomic traits, including genomic signatures and KEGG orthologues (KOs), on microbial environmental responses at both the community and species levels. (4) We also studied the divergent effects of KOs on species environmental responses between rare and dominant species along the gradients of species relative abundance.

following eight isotope–substrate treatments (each with $n = 3$ per ecosystem): $H_2{}^{16}O$ only (16O), 97 atom% $H_2{}^{18}O$ only (18O), ^{12}C-glucose + $H_2{}^{16}O$ (12C), 99 atom % ^{13}C-labeled glucose + $H_2{}^{16}O$ (13C), ^{12}C-glucose + 97 atom% $H_2{}^{18}O$ (12C_18O), ^{12}C-glucose + $H_2{}^{16}O$ + NH_4NO_3 (12C_N), 99 atom% ^{13}C-labeled glucose + $H_2{}^{16}O$ + NH_4NO_3 (13C_N) and ^{12}C-glucose + 97 atom % $H_2{}^{18}O$ + NH_4NO_3 (12C_18O_N) (Fig. 1). After a 7-d

incubation, DNA was extracted from 96 soil samples (four ecosystems × three replicates × eight treatments) and then subjected to ultracentrifugation to separate the DNA according to density along a caesium chloride (CsCl) density gradient. After centrifugation, samples were fractionated, and the DNA in each fraction was purified. The 16S rRNA gene was subsequently quantified via quantitative polymerase chain reaction (PCR) and sequenced using 515F and 806R primers on an Illumina MiSeq instrument within a density range of 1.660–1.735 g/mL (~17 fractions per sample).

Data analysis

Sequence analyses.—The sequences and all accompanying metadata were downloaded from the NCBI SRA database (project PRJNA521534). The raw sequences were analyzed using a custom pipeline. Briefly, FastQC v0.11.8 was used to check the Phred quality of the sequences and the frequency of potential sequencing adapters (Andrews 2010). Then, each sequence was trimmed using the paired-end mode of Trimmomatic v0.39 with an average Phred quality within a four–base pair (bp) sliding window >25, and the filtered reads shorter than 250 bp were discarded (Bolger et al. 2014). Because of the large amount of memory required by the QIIME 2 program (Bolyen et al. 2019), the clustering and selection of operational taxonomic units (OTUs) and taxonomic assignment were achieved using the script 'pick_open_reference_otus.py' in QIIME v1.9.1 (Caporaso et al. 2010). UCLUST (Edgar 2010), a high-speed and low-memory-usage clustering method implemented in QIIME, was used to cluster the sequences with a similarity threshold of 97%. Taxonomic assignment of each OTU was performed using the UCLUST program, which aligns sequences against the Greengenes database (v13.8). OTUs represented by <0.005% of the total sequences were discarded, which results in the inclusion of 2,199 OTUs for the following analyses. Bacterial diversity was estimated as the number of OTUs rarefied to the lowest number of reads (70,000) obtained for each ecosystem replicate. To verify that our results were similar to those in the previous report (Morrissey et al. 2019), nonmetric multidimensional scaling (NMDS) was performed to evaluate the elevational succession of bacterial communities in the 96 samples in the eight treatments (Appendix S1: Fig. S1). These analyses were performed using the R package vegan V2.4.6 (Dixon 2003).

Estimating genomic traits.—We mapped the OTUs corresponding to species whose genome sequence is available in the public database through sequence alignment of the 16S rRNA gene. Although mapping methods based on the alignment of a single gene sequence are controversial in terms of accuracy, similar alignment methods have been widely used and found to produce sound ecological findings (Barberán et al. 2014). In brief, the bacterial and archaeal gbk files available in the NCBI RefSeq database (21,788 species with unique taxonomic identities) were downloaded (date: 2019-04). The 16S rRNA gene sequence (at least 1,000 bp) was extracted from each organism, and the sequences were pooled together to form the reference database. When multiple 16S rRNA genes were present in the genome of an organism, the longest one was selected as the representative. The OTU sequence was aligned against the reference database using the BLASTN program with an e-value of 1e-5. The top hits with a sequence identity larger than 97% and an alignment length >80% longer than the query length were considered closely related representatives of the query OTUs. It should be noted that we used the same similarity threshold 97% in genome matching as our previous 16S rRNA gene sequence analysis. Totally, there are 26.7% OTUs that can retrieve the closely related genomes for the following analyses. For most OTUs identified using this high-throughput amplicon sequencing approach, no closely related genomic sequences were retrieved, partly because of the gap between the sequenced genome in the public database and the extremely high microbial diversity in nature. The corresponding genomic signatures, including genome size, coding DNA sequence number (CDS), total gene number, guanine-cytosine (GC) content, and rRNA operon copy numbers, of the OTUs with matched genome sequences were estimated and assigned to the OTUs. To explore the metabolic potential of the OTUs further, the amino-acid sequences of protein-coding genes in the genomes were aligned against orthologous sequences in the KEGG database (date: 2017-09) using DIAMOND v0.8.22 (Buchfink et al. 2015). The consensus KEGG ortholog identity among the top 10 hits was assigned to the query gene. Unless stated explicitly, the default parameters were used in the programs mentioned above. KO richness was estimated using the number of orthologues for each species. It should be noted, however, that concerns should be taken into account when inferring microbial traits from genomes. For instance, genomes reflect both active and inactive species in natural habitats, like soil, and the relevant findings obtained could be further supported with transcription and translation data. For the better understanding of general ecologists, we used the term "species" to represent "OTU" in the following statistical analyses such as species performance and species environmental response.

Excess atom fraction (EAF) estimation.—The EAFs of ^{18}O and ^{13}C for each species were estimated following the publicly available code associated with qSIP calculations[5] (Hungate et al. 2015, Morrissey et al. 2017). Briefly, a weighted average density (WAD) was calculated for each species' DNA after incubation in the presence of unlabeled and isotopically enriched substrates

[5] https://bitbucket.org/QuantitativeSIP/qsip_repo

(that is, water and glucose) based on its distribution across a CsCl density gradient. The shift in WAD following incubation with an isotopically enriched substrate can be used to quantify the amount of isotope incorporation, based on the theoretically modeled and experimentally verified relationship between isotope incorporation and DNA molecular weight (Hungate et al. 2015). As previously described (Morrissey et al. 2017), tube-level WAD corrections were performed because of the effect of ultracentrifuge tube on species WAD estimation, probably a consequence of slight differences in CsCl density gradients between the tubes.

Estimating species performance with qSIP.—Microbial performance included growth rate (GR) and carbon assimilation rate (CAR), which were measured by the EAFs of ^{18}O and ^{13}C, respectively, at the community and species levels. There were five metrics of microbial performance: the GR without added substrates (GR.na), with added carbon (GR.C) and with added carbon and nitrogen (GR.CN) and the CAR with added carbon (CA.na) and with added carbon and nitrogen (CA.N; Fig. 1). The unlabeled and labeled treatments for estimating the performance metrics of GR.na, GR.C, GR.CN, CAR.na, and CAR.N were "16O vs. 18O," "12C vs. 12C_18O," "12C_N vs. 12C_18O_N," "12C vs. 13C," and "12C_N vs. 13C_N," respectively (Fig. 1).

To scale up species performance to the community level, we calculated the CWM of the species GR (GR_{CWM}) or CAR (CAR_{CWM}), which represents community mean performance based on community composition and reflects the mean species behavior (Fig. 1):

$$\mathrm{CWM} = \sum_{i=1}^{n} p_i \times \mathrm{performance}_i,$$

where p_i is the relative abundance of species i in the community and performance$_i$ is the functional performance value of species i, for example, its GR or CAR. Similarly, CWMs were calculated based on the species environmental responses and the genomic traits of species. This analysis was performed using the R package FD V1.0.12 (Lavorel et al. 2007). Although CWM-based correlations are likely the most widely used approach to link traits to environmental variation, other powerful methods considering both site-level and species-level components such as the fourth-corner approach (Peres-Neto et al. 2017) are encouraged to provide additional supports to current findings in future studies.

Estimating species environmental responses with effect-size qSIP.—Further, to quantify the magnitude and direction (i.e., positive or negative) of microbial responses to environmental alterations such as carbon or nitrogen addition, we proposed an effect-size approach using the standardized mean difference Hedges' g as the effect-size metric (Hedges 1981, Hedges and Olkin 1985):

$$g = \frac{\overline{\mathrm{EAF}}_t - \overline{\mathrm{EAF}}_c}{s_{\mathrm{pooled}}} \times \left(\frac{\Gamma(\mathrm{df}/2)}{\sqrt{\mathrm{df}/2}\Gamma((\mathrm{df}-1)/2)} \right),$$

where $\overline{\mathrm{EAF}}_t$ and $\overline{\mathrm{EAF}}_c$ are the EAF means of the treatment and control groups, respectively, Γ is the gamma function, and $\mathrm{df} = n_t + n_c - 2$. $s_{\mathrm{pooled}} = \sqrt{\frac{\sum(\mathrm{EAF}_t - \overline{\mathrm{EAF}}_t)^2 + \sum(\mathrm{EAF}_c - \overline{\mathrm{EAF}}_c)^2}{n_t + n_c - 2}}$ is the pooled standard deviation of the two groups ($n_t = n_c = 3$).

Hedges' g is a variation of Cohen's d that corrects for biases due to small sample sizes (Hedges and Olkin 1985), which is the case for this study with a replicate of three. Similar to microbial performance, we considered microbial environmental responses at the species and community levels. There were four metrics of microbial environmental responses assessed with Hedges' g: the GR under the addition of carbon (GR.C g), nitrogen (GR.N g), carbon and nitrogen (GR.CN g), and the CAR under nitrogen addition (CAR.N g; Fig. 1). At the bacterial species level, the treatment and control groups of performance metrics used to estimate the response metrics of GR.C g, GR.N g, GR.CN g, and CAR.N g were "GR.C vs. GR.na," "GR.CN vs. GR.C," "GR.CN vs. GR.na," and "CAR.N vs. CAR.na," respectively (Fig. 1). Similarly, for the community level, we calculated the CWM of species environmental responses of GR and CAR (that is, GR_{CWM} g and CAR_{CWM} g, respectively; Fig. 1). We acknowledge there are unstandardized effect-size statistics, such as the unstandardized mean difference between groups, which, however, is not suitable in our study because of the following facts. (1) The standardized mean difference such as Hedges' g allows us to draw more general conclusions for the comparisons across studies, as it has no units and considered both mean difference and the variation within treatment and control groups. (2) High variations could be generated within each group for molecular data such as sequencing and quantitative PCR, which should be standardized by using effect-size Hedges' g when comparing the microbial environmental responses across contrasting ecosystems or upon different nutrient additions. The effect-size analyses were performed using the R package metafor V2.2.8 (Viechtbauer 2010). An example for calculating species environmental response of effect size qSIP are available in Data S1.

Statistical analyses.—We used the following variables, including those related to contemporary environments, bacterial diversity, and genomic traits, as explanatory variables. The contemporary environmental variables included mean annual temperature (MAT) and precipitation (MAP) and soil variables such as soil organic carbon (SOC), soil nitrogen, the C/N ratio, microbial biomass carbon (MBC) and nitrogen (MBN), the MBC/MBN ratio, and soil pH. For genomic traits, we considered genomic signatures (i.e., genome size, CDS, total gene number, GC content, and rRNA operon copy

numbers), KO copy numbers (KOs), and KO richness. The response variables included microbial performance and environmental responses at the community and species levels and were analyzed for their elevational patterns and dependence on contemporary environmental factors and genomic traits.

First, elevational patterns of microbial performance and environmental responses at the community level were visualized using the linear or quadratic model with the lowest Akaike's information criterion value (Sakamoto et al. 1986). We further evaluated the significance of the CWM of microbial performance between performance metrics at each elevation using the Student's *t*-test.

Second, the influences of contemporary environments and genomic traits on microbial environmental responses were evaluated by the Pearson correlation analysis at the community level and by the meta-analysis at the species level.

Pearson correlation analysis was performed to examine the correlations between community-level microbial environmental responses and explanatory variables. Three types of explanatory variables were from three groups: contemporary environments, bacterial diversity, and the CWM of genomic traits. For genomic traits, we considered the CWMs of genome size, CDS, total gene number, GC content, rRNA operon copy numbers, and KO richness; the mean CWM of copy numbers of all KO (mean KOs); and the CWM of copy numbers of each KO (KOs).

Meta-analysis via linear mixed-effects meta-regression models was performed to examine the explanatory power of genomic traits for species environmental responses using the R package metafor V2.2.8 (Viechtbauer 2010). We examined the influences of genomic traits such as genomic signatures and all KOs on species environmental responses for each species and for each bin of species based on their abundances. It should be noted that that assigning genomic traits to the OTUs may differentially affect rare and abundant species, which could bias the results showing different genomic traits between these two groups. To avoid these biases, we studied the relationships between genomic traits and species environmental responses along the gradient of bacterial relative abundance. Specifically, we categorized all species into 22 bins according to their relative abundances and kept species numbers even for all bins. We sequentially removed the species from lower bins and then examined the influence of species abundance on species environmental responses. In each model, we modeled species environmental responses as a function of each of genomic signatures or KOs and used ecosystems (that is, elevations) as random effects. The Wald-type test was used to evaluate model significance, and pseudo-R^2 represented the influence of each KO on the species environmental response. The pattern of this influence across species abundances was modeled using quadratic regression. Further, we examined the influence of Level 2 KEGG pathways of their KOs on species environmental responses. The meta-analytic models were selected using the R package glmulti 1.0.7.1 (Calcagno and de Mazancourt 2010).

Results and Discussion

For the species performance at the community level, GR_{CWM} (that is, community-level growth rate) showed a nonsignificant relationship with elevation without environmental alteration ($P = 0.159$) (Fig. 1a). When carbon was added, GR_{CWM} significantly increased at the two lower elevations (*t*-test, $P < 0.05$) and showed marginally significant decreases with increasing elevation ($P = 0.083$; Fig. 2a). Nitrogen addition further promoted GR_{CWM} at higher but not lower elevations (Fig. 1 a). In contrast, CAR_{CWM} (that is, community-level carbon assimilation rate) increased upon nitrogen addition at low and high elevations (*t*-test, $P < 0.05$) and showed hump-shaped elevational patterns, with the lowest values at the highest elevation, regardless of nitrogen addition (Fig. 2a).

For the microbial environmental response at the community level, nitrogen addition generally substantially improved GR_{CWM} *g* (that is, community-level growth rate response) at high elevations, leading to significant increases toward high elevations, regardless of whether carbon was included ($P = 0.008$ and $P < 0.001$ for GR.CN *g* and GR.N *g* in Fig. 2b, respectively). In contrast, CAR_{CWM} *g* (that is, community-level carbon assimilation response) under nitrogen addition showed no such pattern ($P = 0.565$; Fig. 2b). These increases in GR_{CWM} *g* with increasing elevation clearly indicated stronger nitrogen limitation towards higher elevations, which was consistent with the previous comparison of GR_{CWM} between carbon and carbon-nitrogen addition treatments revealing strongly promoted microbial growth upon nitrogen addition at higher elevations (Fig. 2a). Such nitrogen limitation at high elevations was further confirmed by the coordinated differences in GR_{CWM} *g* between nitrogen and carbon–nitrogen additions (that is GR.N *g* and GR.CN *g* in Fig. 2b, respectively). These findings also revealed little variation in the effects of carbon on GR_{CWM} *g* across drastically different ecosystems, which was also supported by the nonsignificant elevational patterns of GR_{CWM} *g* with carbon addition alone (that is GR.C *g* in Fig. 2b; $P = 0.293$). Our findings of nitrogen limitation, also indicated by larger soil C/N ratio than MBC/MBN ratio, revealed the effects of soil nitrogen and carbon stoichiometry underlying growth-rate variations of microbial communities across elevations.

This unbalanced stoichiometry of nitrogen and carbon was further supported by Pearson correlations between contemporary environments and the community-level responses of GR or CAR. For instance, the GR_{CWM} *g* under nitrogen and carbon–nitrogen additions, but not the value under carbon addition, were most highly

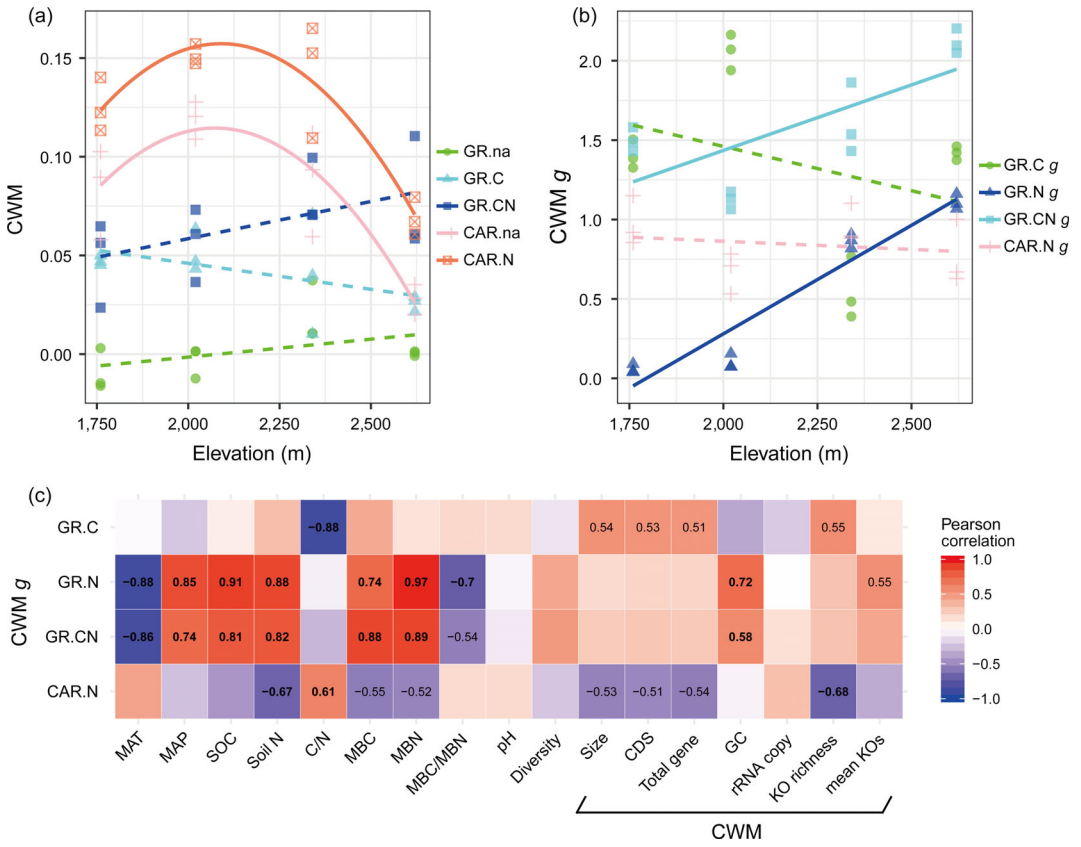

FIG. 2. Community-level species performance (a) and environmental responses (b) to nutrient additions and the influences of environmental factors and genomic traits (c). (a) These community-level measures were evaluated with community-weighted means (CWMs) of two measures of species performance (growth rate [GR] and carbon assimilation rate [CAR]) and then plotted against elevation. For GR, three conditions were considered: without added substrates (GR.na), with added carbon (GR.C) and with added carbon and nitrogen (GR.CN). For CAR, there were two conditions: with added carbon (CAR.na) and with added carbon and nitrogen (CAR.N). (b) The species environmental responses were quantified by the effect size metric Hedges' g. The community-level responses of GR (GR_{CWM} g) were assessed for three scenarios: the addition of carbon (GR.C g), nitrogen (GR.N g), and carbon and nitrogen (GR.CN g). The community-level responses of CAR (CAR_{CWM} g) were evaluated only under nitrogen addition (CAR.N g). (c) The heat map shows the positive (red) and negative (blue) Pearson coefficients between microbial environmental responses and explanatory variables. Explanatory variables are from three categories: contemporary environments, bacterial diversity, and CWMs of genomic traits. The contemporary environmental variables include mean annual temperature (MAT) and precipitation (MAP), and soil variables such as soil organic C (SOC), soil N, the C/N ratio, microbial biomass carbon (MBC) and nitrogen (MBN), the MBC/MBN ratio and soil pH. For genomic traits, we considered the CWMs of genome size, coding DNA sequence number (CDS), total gene number, GC content, rRNA operon copy numbers and KEGG orthologue (KO) richness, as well as the mean CWM of KO copy numbers (mean KOs). The bold and regular numbers in the heat map indicate the Pearson coefficients with $P < 0.05$ and $P < 0.1$, respectively.

positively associated with MBN, with Pearson r values of 0.97 and 0.89, respectively ($P < 0.001$; Fig. 2c). This pattern suggests that nutrient additions including nitrogen promoted bacterial growth and that the microbes having high nitrogen demand were more responsive to nitrogen than carbon additions. This result was further supported by the negative Pearson r value between MBC/MBN and GR_{CWM} g under nitrogen and carbon–nitrogen additions, which were −0.70 and −0.54, respectively ($P = 0.01$ and 0.07, respectively; Fig. 2c). Further, the positive GR responses to nitrogen additions were supported by the nitrogen demand exceeding its supply, which was revealed by the fact that the GR_{CWM} g under carbon addition, but not the values under nutrient additions including nitrogen, was strongly negatively related to soil C/N ratio ($r = -0.88$, $P < 0.01$; Fig. 2c), indicating the deteriorated nitrogen supply upon alleviated carbon limitation. Nitrogen limitation was also supported by CAR_{CWM} g under nitrogen addition alone, which showed a significant and strong positive relationship with the soil C/N ratio ($r = 0.61$, $P = 0.03$) but no relationship with MBC/MBN ($r = 0.18$, $P = 0.58$; Fig. 2c).

In addition to the contemporary environments considered above, genomic traits were also an important driver of microbial community responses to nutrient additions (Fig. 2c). However, these traits were rarely examined in previous literature, especially at the species level. For

example, the effects of genomic traits on CAR_{CWM} *g* under nitrogen addition were revealed by its significant negative correlations with the CWMs of genome size, total gene number, and KO richness (Kanehisa et al. 2016; $r = -0.51$ to -0.68, $P = 0.01 \sim 0.09$; Fig. 2c). This finding was further supported by a proportion (12.0%) of KOs that were significantly correlated with CAR_{CWM} *g* under nitrogen addition, most of which showed negative correlations (Fig. 3a, b). Further, compared to that under carbon addition, the GR_{CWM} *g* under nitrogen and carbon–nitrogen additions was more strongly explained by and positively associated with the average CWM of KO copy numbers and the CWM of GC content ($r = 0.55$–0.72, $P = 0.009$–0.06; Fig. 2c). This result may have been caused by increased growth responses due to the enhanced metabolism upon nutrient additions including nitrogen. This finding was further supported by three observations. (1) There were higher percentages of KOs that were significantly correlated with GR_{CWM} *g* upon nitrogen addition (hereafter defined as significantly responsive KOs), that is, 47.3% and 38.1% for nitrogen and carbon–nitrogen additions, respectively (Fig. 3a). (2) These significantly responsive KOs were from more KEGG pathways which are related to multiple biosynthesis and energy-yielding processes, such as amino acid metabolism, carbohydrate

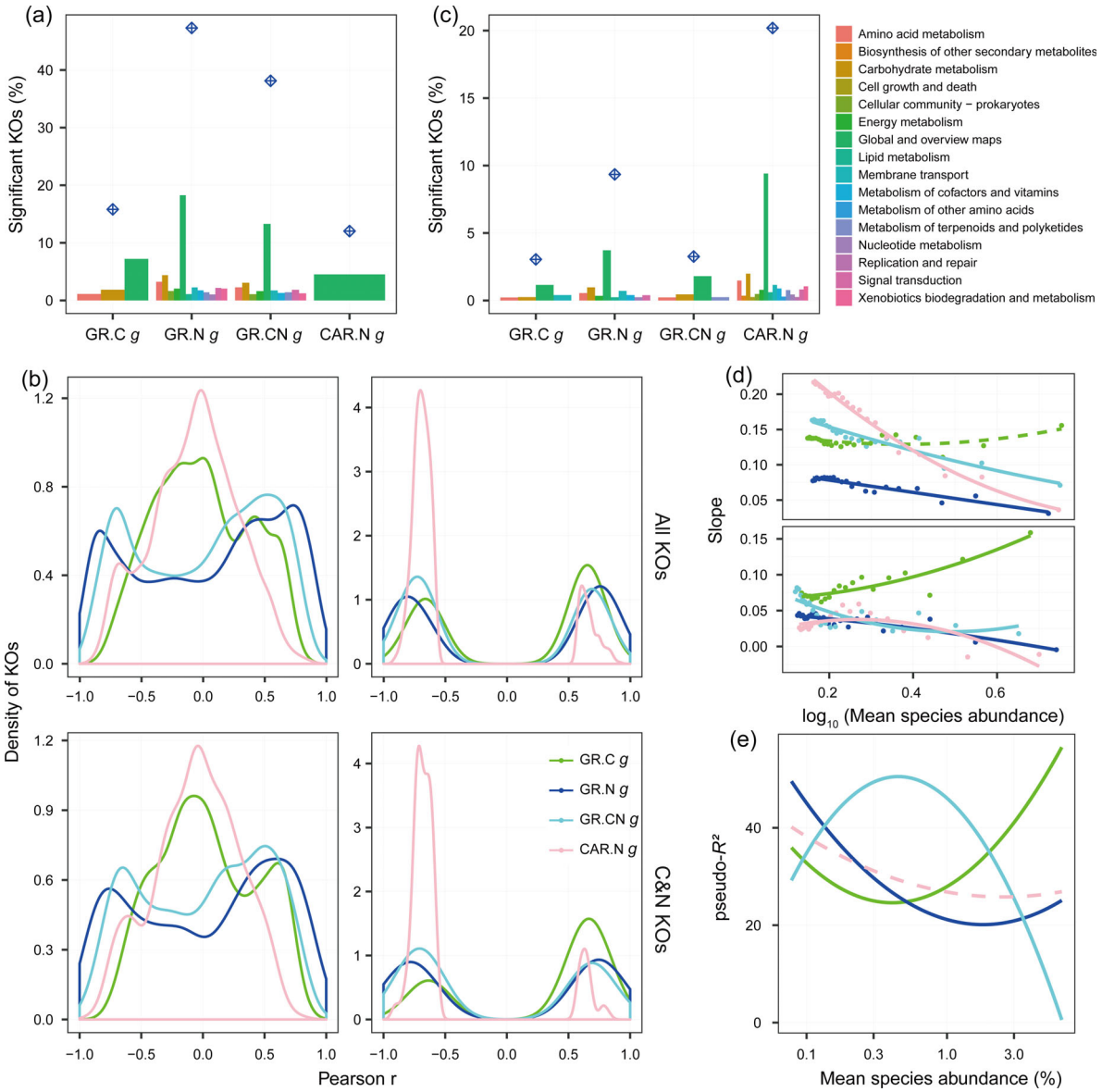

FIG. 3. The influences of genomic traits on microbial environmental responses at the community and species levels. There were four metrics of microbial environmental responses assessed with Hedges' *g*: the growth rates (GR) under the addition of carbon

(FIG. 3. *Continued*)

(GR.C *g*), nitrogen (GR.N *g*), and carbon and nitrogen (GR.CN *g*) and the carbon assimilation rate (CAR) under nitrogen addition (CAR.N *g*). (1) At the community level, the influences were examined by Pearson correlation analyses for all KOs (a), (b). (a) For each response metric, the blue rectangular point indicates the percentage of KOs that were significantly ($P < 0.05$) correlated with the community-level responses. For better visualization, we show only 12 KEGG pathways, each of which has >1% of significantly responsive KOs, as shown in the figure legend. (b) The density plot shows the Pearson coefficient distribution for the KOs involved in all KEGG pathways (all KOs; upper panel) and the KOs involved in carbon- and nitrogen-related pathways (C&N KOs; lower panel) for each response metric. The carbon- and nitrogen-related pathways include carbohydrate metabolism and energy metabolism. (2) At the species level, the influences were examined using linear mixed-effects meta-regression models and the meta-analysis framework (c)–(e). (c) We examined the influences of all KOs on the responses of each species. For each response metric, the blue rectangular point indicates the percentage of KOs that were significantly ($P < 0.05$) correlated with the species-level responses. For better visualization, we show only 16 KEGG pathways, each of which has >0.2% of significantly responsive KOs, as shown in the figure legend. (d) The influences of species abundance on positive (upper panel) or negative (lower panel) responses along the species abundance gradient. The species were categorized into 22 bins according to their relative abundances, with even species numbers for all bins. These bins were numbered from 1 to 22, and the higher number indicates higher relative abundance. We sequentially removed the species from lower bins and then examined the influence of species abundance on species-level responses by linear model for positive or negative species environmental responses (that is, Hedges' *g*). The influence was quantified by the slope of abundance–response relationships with linear model, as detailed in Appendix S1: Fig. S8. For these influences along the species-abundance gradient, solid lines are the significant ($P < 0.05$) quadratic regression fits, and dotted lines indicate non-significant relationships ($P > 0.05$). Mean species abundance is the average of species relative abundance for each scenario as indicated in Appendix S1: Fig. S8. (e) The influences of KOs on species environmental responses across the 22 species-abundance–based bins. The influence for each bin (that is, the explained variation, %) was quantified by pseudo-R^2 by considering only the KOs that had highly significant Pearson coefficients with microbial environmental responses at the community level (that is, the top 50 KOs, $P < 0.001$). The patterns of influence along the species abundance gradient are indicated by solid lines obtained using quadratic regression models ($P < 0.001$ for GR.C *g* and GR.CN *g*, $P = 0.025$ for GR.N *g*, and $P = 0.080$ for CAR.N *g*). Mean species abundance is the averaged species relative abundance for each bin (e).

metabolism, energy metabolism, membrane transport, and cofactor and vitamin metabolism (Fig. 3a). (3) These significantly responsive KOs showed stronger correlations with GR_{CWM} *g* under nitrogen and carbon–nitrogen additions for either the KOs involved in all KEGG pathways (Fig. 3b, Appendix S1: Fig. S2), or the KOs involved in carbon- and nitrogen-related pathways, such as carbohydrate and energy metabolism (Fig. 3b; Appendix S1: Fig. S3) and nitrogen metabolism (Appendix S1: Fig. S4) pathways.

These community-level findings suggest that genomic traits may also explain species-level responses to environmental changes. When all species were considered, there were significant relationships between such responses and genomic traits, as expected at the species level (Fig. 3c; Appendix S1: Fig. S5, S6). For instance, compared to that under carbon addition, the three following observations were made for the Hedges' *g* of the GR or CAR, particularly upon nitrogen addition alone: (1) Weaker associations with genomic signatures such as genome size and total gene number (Appendix S1: Fig. S5). (2) Higher percentages of significantly responsive KOs; that is, 9.3% and 20.2% for the *g* of GR and CAR with nitrogen addition, respectively (Fig. 3c). (3) Higher diversity in the KEGG pathways of these significantly responsive KOs (Fig. 3c); and (4) higher percentages of the lower-level pathways associated with significantly responsive KOs in multiple top-level pathways, such as glycan biosynthesis and metabolism, metabolism of cofactors and vitamins, and replication and repair (Appendix S1: Fig. S6).

Interestingly, preliminary patterns in the volcano plot revealed that many dominant species showed neutral responses to nutrient resource changes, indicated by low Hedges' *g* values (Appendix S1: Fig. S7a). These phenomena collectively indicate that rare and dominant species may respond differently, as previous literature has suggested (Lynch and Neufeld 2015). Thus, we categorized the species into 22 bins according to their relative abundances (Appendix S1: Fig. S7b) and quantified the abundance–response relationship of each bin with the slope of linear model for negative or positive species environmental responses. We found that the slope increased toward higher species abundances under carbon addition, but declined under nutrient additions including nitrogen ($P < 0.001$; Fig. 3d; Appendix S1: Fig. S8).

Further, to integrate the effects of genomic traits on the negative and positive responses along the species abundance gradients within a united data-analysis framework, we conducted meta-analyses with linear mixed-effects meta-regression models. We explored the correlations between species environmental responses and genomic traits for each bin (Fig. 3e; Appendix S1: Fig. S9). Specifically, when considering only the KOs with highly significant Pearson coefficients with microbial environmental responses at the community level (that is, the top 50 KOs; $P < 0.001$), we found that under carbon addition the pseudo-R^2 between these KOs and the species-level GR *g* showed a rising right-hand side of U-shaped pattern, with the highest values at the highest species abundances ($P < 0.001$; Fig. 3e; Appendix S1: Fig. S9a). Interestingly, under nitrogen addition alone such influences of KOs on species environmental responses showed a sinking left-hand side of the "U," with the highest values at the lowest species abundance

($P < 0.001$), but a hump-shaped pattern appeared under carbon–nitrogen addition with the highest pseudo-R^2 at ~0.5% of species abundance ($P < 0.001$; Fig. 3e; Appendix S1: Fig. S9a). Similar patterns were observed under these nutrient additions when all KOs were considered (Appendix S1: Fig. S9b). These results collectively indicate that the associations between genomic traits and species environmental responses were strongest for the dominant species under carbon addition, and strongest for the rare species under nitrogen addition. Such distinct associations probably occurred because rare and common bacteria are implicated in fundamentally different types of ecosystem functioning.

In summary, we developed a new approach named effect-size qSIP to quantify microbial species performance responses to environmental changes and further applied it to measure species environmental responses upon nutrient additions for soil bacterial species in diverse ecosystems along an elevational gradient. The findings revealed by this approach have at least three substantial implications regarding species- and community-level responses to environmental changes.

1) Nitrogen limitation on community-level GR changes at high elevations indicates the high vulnerability of ecosystem carbon storage and stabilization in response to global changes such as anthropogenic nitrogen deposition, especially in nitrogen-poor regions, such as arctic regions, or at high elevations (Du et al. 2020).
2) In addition to contemporary environments, genomic traits are extremely important for the changes in microbial GRs and CARs in response to environmental changes at both the community and species levels. Modeling soil carbon dynamics in response to environmental changes by explicitly considering microbial functional traits can increase model accuracy (Hagerty et al. 2018, Sulman et al. 2018). It is thus essential to integrate microbial genomic traits into models such as the microbial–mineral carbon stabilization (MIMICS; Wieder et al. 2015) and the decomposition model of enzymatic traits (DEMENT; Allison 2012, Allison and Goulden 2017, Malik et al. 2020) models to improve predictions of global carbon fluxes in response to future environmental changes.
3) Taking advantage of this new approach at the species level, we revealed for the first time that species environmental responses under carbon and nitrogen additions are more strongly associated with essential traits for rare and dominant species, respectively. This phenomenon indicates that rare and dominant species differentially respond to nutrient enrichment via their metabolic traits. Further studies are encouraged to test this generality with diverse taxonomic groups and species performance in response to other environmental changes, such as climate warming, phosphorus-driven eutrophication, and anthropogenic disturbances.

Taken together, our new approach with stable isotope labeling of DNA is generally applicable in natural complex environments such as soil and aquatic ecosystems in a biogeographic context to evaluate the response of microbial species performance to environmental changes quantitatively and further the underlying mechanisms at the species level. The obtained insights can lead to a more holistic understanding of microbial environmental responses in natural environments, which will be essential for predicting microbial community responses to global environmental changes.

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Supporting Information

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