

## LETTER

# Stress causes interspecific facilitation within a compost community

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**Abstract**

Ecological theory predicts interactions between species to become more positive under abiotic stress, while competition should prevail in more benign environments. However, experimental tests of this stress gradient hypothesis in natural microbial communities are lacking. We test this hypothesis by measuring interactions between 10 different members of a bacterial community inhabiting potting compost in the presence or absence of toxic copper stress. We found that copper stress caused significant net changes in species interaction signs, shifting the net balance towards more positive interactions. This pattern was at least in part driven by copper-sensitive isolates – that produced relatively small amounts of metal-detoxifying siderophores – benefitting from the presence of other species that produce extracellular detoxifying agents. As well as providing support for the stress gradient hypothesis, our results highlight the importance of community-wide public goods in shaping microbial community composition.

**KEYWORDS**

abiotic stress, bacteria, commensalism, community, mutualism, public goods, species interactions, toxic metals

**INTRODUCTION**

Bacteria are embedded within complex communities, where they face a constant struggle for limiting resources (Foster & Bell, 2012; Ghoul & Mitri, 2016; Hibbing et al., 2010). Ecological theory predicts that, while competitive interactions prevail in permissive environments, abiotic stress should favour more positive interactions (Stress Gradient Hypothesis: Bertness & Callaway, 1994). Two general non-mutually exclusive mechanisms could underpin a shift towards positive interactions in response to abiotic stress. First, stress-mediated population size reductions are likely to alleviate the magnitude of resource and/or interference competition between species

(Chesson, 2000). Second, abiotic stress could promote facilitative interactions (Bruno et al., 2003), wherein both (mutualism: Bronstein, 1994) or one (commensalism: Mathis & Bronstein, 2020) of the species involved derive a benefit without causing harm to either. Key examples of facilitative interactions in bacterial communities include the extracellular breakdown of toxins (Estrela et al., 2019) and syntrophy, where species cross-feed on waste products produced by others (D'Souza et al., 2018). Mounting evidence shows that the functioning and stability of bacterial communities are greatly affected by such facilitative interactions (Cavaliere et al., 2017; Coyte et al., 2015; Rodríguez-Verdugo & Ackermann, 2020).

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Support for the Stress Gradient Hypothesis (SGH) from observational studies is very mixed (Bowker et al., 2010; Bruno et al., 2003; He et al., 2013; Li et al., 2013) and the underlying mechanisms remain often unclear (Lawrence & Barraclough, 2015). This highlights the need for well-controlled experimental studies to fill this important knowledge gap. A recent experimental test reported more positive interactions under toxic environmental conditions in simple *in vitro* bacterial communities (Piccardi et al., 2019). In this model system, some species detoxified metal working fluid to the benefit of the rest of the community (Piccardi et al., 2019), thereby masking underlying competitive interactions. However, the mechanisms responsible for the observed increase in facilitation remain unknown. To explore the generality and ecological relevance of this finding, we investigated whether toxic metal stress in a semi-natural environment (potting compost) increased the extent of positive interactions between members of the resident bacterial community.

Both the competition–alleviation and the facilitation–opportunity mechanisms are likely to operate in metal-polluted bacterial communities. First, metals typically reduce bacterial population sizes (Chen et al., 2014; Gadd & Griffiths, 1977), thereby potentially reducing interspecific competition (Lawrence & Barraclough, 2015). Second, extracellular detoxifying agents, such as siderophores, could potentially provide protective benefits and ameliorate toxic metal conditions. Siderophores are small, metal-chelating compounds that are secreted by many microbes (Hider & Kong, 2010; Ratledge & Dover, 2000). These extracellular molecules are perhaps best known for their function as iron carriers, but siderophores are often up-regulated in response to metal toxicity: siderophores are released into the environment, where they bind to toxic metals and prevent them from being taken up and killing cells (Schalk et al., 2011); hence, detoxification takes place outside the bacterial cell. Importantly, siderophores not only protect the producer from toxic metal stress, but potentially also conspecifics (Braud et al., 2010; O'Brien et al., 2018) and other community members (Hesse et al., 2018). In particular, using the model species *Pseudomonas aeruginosa*, we have previously shown that heterospecific siderophores can rescue an isogenic siderophore knockout mutant (so-called ‘cheats’) from toxic copper stress (Hesse et al., 2018).

Investment in siderophores is, therefore, likely a key phenotypic trait shaping the outcome of species interactions (Kramer et al., 2019). If siderophores indeed facilitate the growth and survival of metal-sensitive taxa, then this might shift the balance towards more positive interactions. However, this facilitation might then increase competition between the beneficiary species and the producer leading to more negative interactions and even competitive exclusion (Chesson, 2000). The beneficiary species could be considered as ‘cheats’ in this

context, with cheating species stably maintained in metal-polluted environments because siderophore production can be metabolically costly (Sexton & Schuster, 2017).

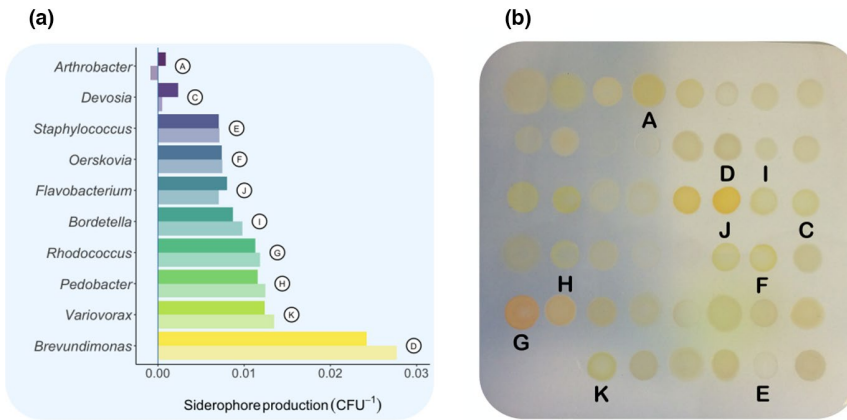
To test whether abiotic stress causes an increase in the prevalence of positive interactions, we exposed bacteria to toxic copper – an ecologically and economically important density-independent stressor (Gadd, 2010). We conducted pairwise competitions among 10 random compost isolates, as well as growing each in isolation over 7 days in copper-polluted or unpolluted compost. We determined net interaction signs by comparing the population densities of partners when grown alone versus together. In line with the SGH, the net balance of interactions shifted towards more positive interactions in response to copper stress, with metal-sensitive, low siderophore-producing bacterial taxa benefitting most from the presence of other species.

## MATERIAL AND METHODS

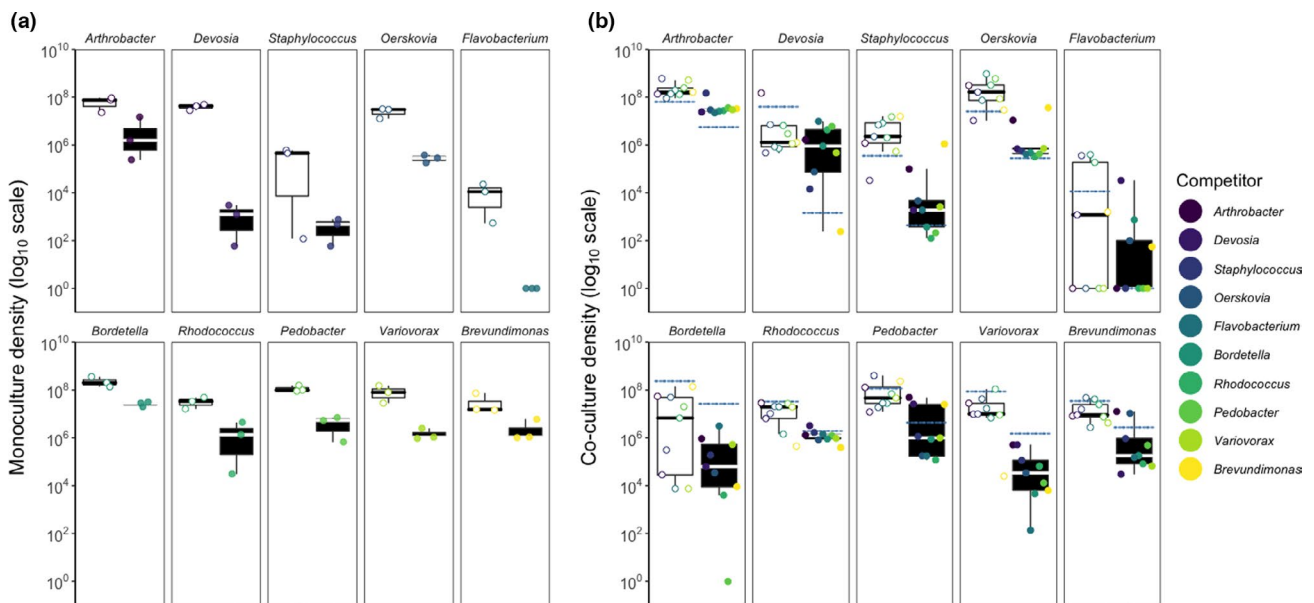
### Pairwise competition assays among compost isolates

We carried out pairwise competition assays among 10 random compost isolates. We previously isolated bacterial taxa from experimental compost microcosms to test how the presence of toxic metals affects community-wide siderophore production (Hesse et al., 2018). Briefly, bacteria were isolated by plating soil washes on King's B (KB) agar, after which individual colonies were randomly picked and Sanger sequenced to determine genus-level identity ( $n = 576$  isolates in total). We selected a subset of 10 isolates, ensuring these varied widely in their colony morphology, size and siderophore production (Figure 1). Colony morphologies were stable in our compost microcosms throughout the course of the experiment. We also validated colony identity for a subset of isolates using Sanger sequencing. Together, this enabled us to distinguish and quantify colonies of all different isolates in pairwise co-cultures.

Before each experiment, each of the 10 isolates was independently grown for 48 h (26°C, 200 rpm) in glass vials (25 ml) containing 6 ml of KB broth, starting from a single colony. Cultures were spun down and washed twice, before cells were eluded and diluted in M9 salt solution to standardise inoculation density across isolates. Monocultures of each isolate were established in triplicate by inoculating  $\sim 2 \times 10^5$  colony-forming units (CFUs) in glass vials, each containing 5 g of twice-autoclaved sterile compost (John Innes No. 2). Starting population sizes were quantified through plating on KB agar. Using a similar approach, we established all possible pairwise competitions ( $n = 45$ ) by co-inoculating  $\sim 10^5$  CFUs per isolate in duplicate vials, keeping total inoculation densities constant across environments. Compost



**FIGURE 1** Compost isolates used in the pairwise competition assays. Bacteria were previously isolated from a natural compost community (Hesse et al., 2018). For this study, we selected a subset of 10 phylogenetically diverse isolates that varied in their siderophore production (a) and colony morphology (b). The bar plot in panel (a) depicts density-corrected variation in siderophore production, which was measured in duplicate for each isolate. Biological replicates are depicted in different shades, and colours correspond to different isolates. We used mean siderophore production per isolate as explanatory variable in subsequent analyses. Panel (b) shows variation in colour when isolates were plated on King's B agar. Letters underneath spotted cultures depict the subset of isolates used, and their corresponding full genus names are provided in panel (a). Combined with information on colony size and opacity, this enabled us to distinguish competitors during pair-wise competition



**FIGURE 2** The impact of copper stress on bacterial population densities. Boxplots showing the effect of toxic copper on final population densities (CFU g<sup>-1</sup> soil) in (a) monoculture and (b) co-culture. Each subpanel shows isolate-specific variation in population density as a function of copper stress, where white and black boxes depict unpolluted and copper-polluted compost respectively. Boxes depict the upper and lower quartiles of the treatment-specific raw data with the centre line showing the median and whiskers providing a measure of the 1.5× interquartile range. Coloured dots in panel (b) summarise the mean effect of different competitors on focal densities, and horizontal dashed lines depict treatment-specific mean monoculture density

microcosms were incubated statically at 26°C and 75% humidity for a total of 7 days. This equated to a maximum of 10 population doublings (1000-fold increase in population size), minimising any potential impact of evolutionary change. After 24 h of growth, half of the compost microcosms received a single dose of toxic copper sulphate (0.4 ml of 0.25 M CuSO<sub>4</sub>), which reduced bacterial densities without causing large-scale extinctions (with the exception of *Flavobacterium*: see Figure 2). The

remainder of the microcosms received an equal volume of sterile ddH<sub>2</sub>O. At the end of the incubation period, freezer stocks were prepared by vortexing the compost microcosms for 1 min with 15 ml of M9 buffer and sterile glass beads, after which the supernatant was stored at -80°C in a final concentration of 25% glycerol. Serial diluted freezer stocks were then plated on KB agar and individual colonies were identified and counted to obtain estimates of population density (CFU g<sup>-1</sup> soil).

We assayed siderophore production of each taxon in isolation in a common garden environment using individual liquid CAS assays on cell-free supernatants (Harrison et al., 2008; Schwyn & Neilands, 1987). Briefly, isolates were grown individually in 6 ml of KB broth in duplicate for 48 h at 26°C, after which 100 µl of cell-free filtered supernatant was assayed for the extent of iron chelation by mixing supernatant at a 1:1 ratio with CAS solution. Siderophore production was then estimated using:  $(1 - (A_i/A_{ref})) / (CFU_i)$ , where  $CFU_i$  = the number of CFUs in 100-µl culture and  $A_i$  = absorbance at 630 nm of the assay mixture (culture + CAS) and  $A_{ref}$  = absorbance at 630 nm of reference mixture (KB + CAS) (Harrison et al., 2008). For some samples, absorbance reads were negative following reference correction so we standardised siderophore production by setting the minimum observed value to zero. CAS assays performed in iron-limited KB medium (supplemented with 20 mM NaHCO<sub>3</sub> and 100 µg ml<sup>-1</sup> human apotransferrin) provided qualitatively similar results (linear relationship:  $y = -0.017 (-0.024, -0.009) + 1.325 (0.85, 1.799) \times x$ ; adjusted  $R^2 = 0.82$ ). Note that it was not possible to measure siderophore production *in situ*, because the CAS assay is interfered by the presence of natural metal chelators in compost.

## Data analyses

To confirm that the level of copper we used imposed abiotic stress, we used a generalised linear mixed effects model (GLMM) (`glmmTMB()` function in the ‘`glmmTMB`’ package; Brooks et al., 2017) with final population size ( $N_{monoculture}$ ) as response variable, copper treatment as fixed effect and random intercepts fitted for individual isolates. We used a negative binomial error distribution with a log-link function (Harrison et al., 2018; Zuur et al., 2010) and checked residual behaviour using the ‘`DHARMA`’ package (Hartig, 2020). Based on the obtained simulation-based residual plots, we included isolate-specific dispersion parameters.

To determine the effect of copper on co-culture densities, we used a GLMM with a zero-inflated negative binomial error distribution, copper as explanatory variable and dispersion parameters fitted for individual isolates. To model the dependency of paired observations, we fitted additive random intercepts for individual pairs ( $n = 180$ ) and isolates ( $n = 10$ ).

We next determined the sign of species interactions by calculating differences in final population sizes ( $N$ ) in co-culture versus monoculture for each isolate:  $d = \log_2(N_{co-culture} / \bar{N}_{monoculture})$ . We defined interactions as negative if  $d < 0$  or positive if  $d > 0$ . To visualise how copper stress affects the nature of species interactions, we plotted pairwise interactions in a linear

network using the R ‘`igraph`’ (Csardi & Nepusz, 2006) and ‘`ggraph`’ (Pedersen, 2020) packages, selecting interactions with  $|d| > 1.25$  (averaged across duplicates). Arrow thickness in these networks is proportional to  $d$ .

To test whether copper stress changed the composition of species interactions, we carried out a linear mixed model (LMM) (`lmer()` function in the ‘`lme4`’ package; Bates et al., 2015) on  $d$ , with copper treatment as explanatory variable. Again, we fitted random additive intercepts for pairs and isolates. To improve residual behaviour, we transformed  $d$  using an inverse hyperbolic sine transformation in this and subsequent models. To investigate into more detail which isolates showed significant net changes in interaction sign in response to copper stress, for each isolate we carried out one-sample t-tests by comparing  $\Delta d = \bar{d}_{copper, competitor_i} - \bar{d}_{control, competitor_i}$  to zero, corrected for multiple testing (‘`fdr`’ method).

To determine whether siderophores are important predictors of species interactions, we first confirmed siderophores protect isolates from copper stress when grown as monocultures. We fitted a generalised linear model (GLM with Gaussian error structure) with siderophore production as explanatory variable and between-treatment differences in monoculture density as response variable:  $\Delta N = \log_2(\bar{N}_{copper} / \bar{N}_{control})$ , which is more negative for isolates that are more sensitive to copper stress.

We next wanted to examine whether there was direct siderophore facilitation and/or exploitation. We carried out a LMM on  $d$  (i.e. our measure of species interaction sign and strength), with copper and siderophore production of the focal isolate as explanatory variables, as well as their interaction. Again, we fitted random additive intercepts for pairs and isolates. If there was exploitation, then  $d$  should be negative for isolates producing relatively large amounts of siderophores, and positive for non-producing isolates who can reap the detoxifying benefits of siderophores produced by others.

We finally examined whether copper-sensitive isolates received greater protective benefits from benefactors that produced relatively large amounts of siderophore. We used the LMM framework as above, but replaced siderophore production of the *focal* isolate with that of the *partnered* isolate. Copper-mediated changes in the slope would indicate biased facilitation, where partners with, for example, greater investment in siderophores would provide greater protection.

For all analyses, the most parsimonious model was arrived at by sequentially deleting terms and comparing model fits using Chi-squared tests, after which pairwise contrasts were computed using the ‘`emmeans`’ packages (Lenth, 2016), with  $\alpha < 0.05$ . Parameter estimates and their confidence intervals were computed using the ‘`parameters`’ package (Lüdtke et al., 2020). All plots were produced using the ‘`ggplot2`’ package (Wickham, 2016). We used R Version 4.0.3 for all analyses (R Development Core Team; <http://www.r-project.org>).

## RESULTS

### Copper stress changes the nature of species interactions

To test the effect of abiotic stress on the nature of species interactions, we grew monocultures and co-cultures in compost with or without copper stress over 7 days. Despite isolate-specific variation in base line densities (estimated  $\sigma_{\text{isolate}} = 0.93$  and  $1.40$  for monocultures and co-cultures respectively), copper stress significantly reduced bacterial densities when isolates were grown as both monocultures and co-cultures (GLMM: effect of copper on  $N_{\text{monoculture}}$ :  $\chi_1^2 = 55.09$ ,  $p < 0.01$ ;  $N_{\text{co-culture}}$ :  $\chi_1^2 = 117.58$ ,  $p < 0.001$ ; Figure 2).

We next calculated the strength and sign of species interactions by comparing mono- and co-culture densities. We found that interactions in unpolluted compost were, on average, negative: population densities were greater for isolates grown as monocultures compared with co-cultures ( $\bar{d} \pm 95\%$  confidence intervals =  $-0.72$  [ $-1.5, 0.07$ ]; Figure 3a and Figure S1). We next used a network approach to summarise species interactions. In these networks, nodes depict the different isolates, which are connected by arcs that summarise the interaction strength (different weights) and sign (different colours: dark and light blue depict positive and negative interactions respectively) between different pairs. We found that copper pollution significantly altered the composition of species interactions (LMM: effect of copper on  $d$ :  $\chi_1^2 = 8.99$ ,  $p < 0.01$ ), with interactions becoming more positive under copper stress (compare dark blue lines in Figure 3b,c). As a result, interactions were, on average, net neutral in the presence of copper ( $\bar{d} \pm 95\%$  CI =  $-0.23$  [ $-1.02, 0.55$ ]).

To explore these changes in species interactions resulting from copper stress in more detail, we examined which isolates displayed a significant net change in interaction sign by comparing  $d$  in copper-polluted versus unpolluted compost for each unique species pair. We found that three species showed a net positive change (one-sample  $t$ -tests comparing  $\Delta d$  to zero: *Arthrobacter* ( $t_8 = 3.46$ ,  $p_{\text{adj}} < 0.001$ ), *Devosia* ( $t_8 = 8.21$ ,  $p_{\text{adj}} < 0.001$ ) and *Flavobacterium* ( $t_8 = 3.74$ ,  $p_{\text{adj}} < 0.01$ ); Figure 3d), while no species showed a net negative change (all  $p_{\text{adj}} > 0.05$ ; Table S1 & Figure 3d). Together, these findings lend support for the SGH.

### Potential mechanisms underpinning shifts in the nature of species interactions

Two non-mutually exclusive mechanisms could underpin the net positive change in interaction sign observed in these three species. First, the lower population sizes in the presence of copper (Figure 2) likely reduced competition between isolates, leading to less negative

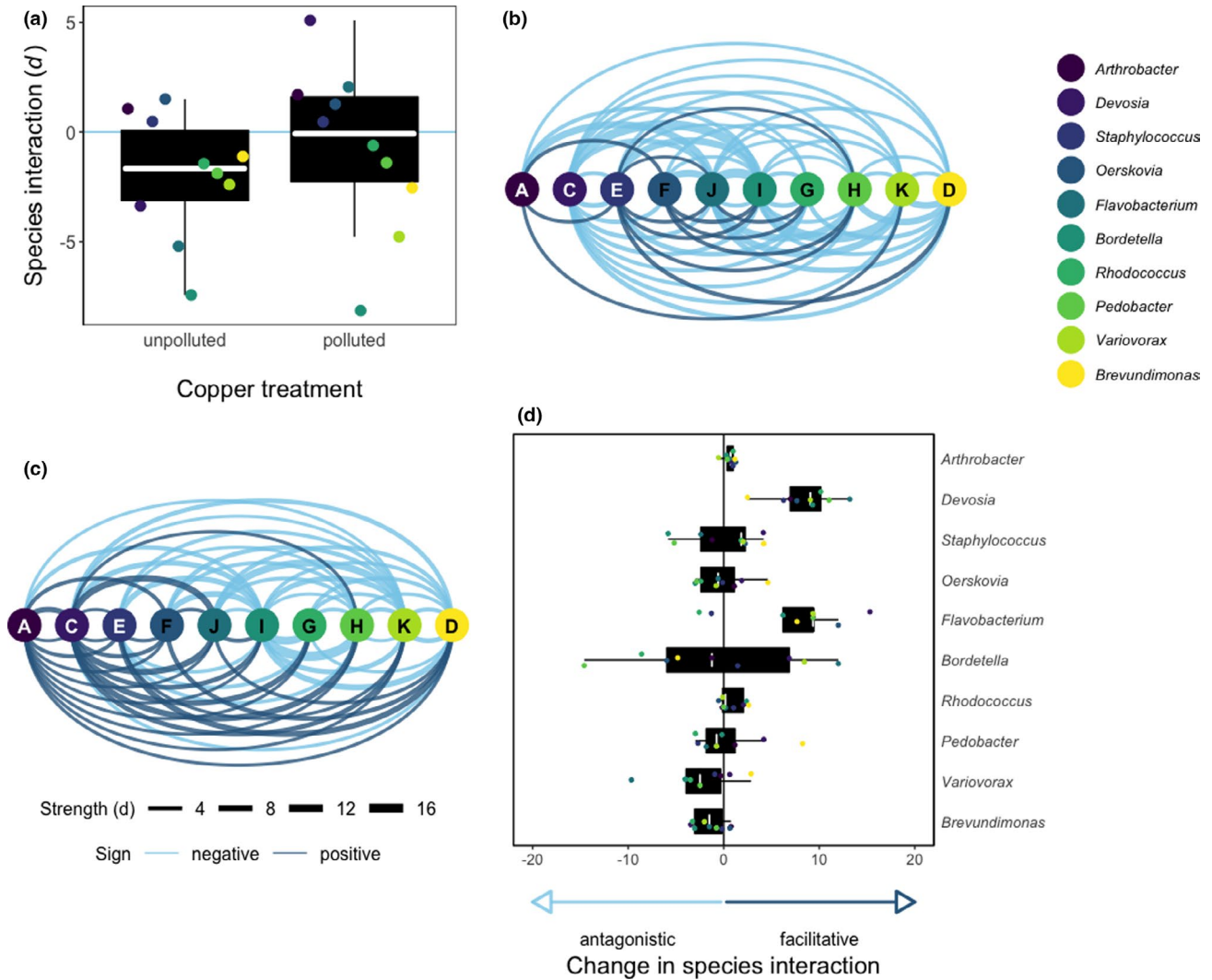
interactions. However, in the cases where copper caused significant changes in interaction sign, these interactions were absolute positive, as opposed to simply being less negative (Figure 3a). This suggests a more important role of the second mechanism: facilitation of metal-sensitive isolates by other species' extracellular detoxification of the environment.

Siderophores can detoxify metal-contaminated environments (Hesse et al., 2018; Schalk et al., 2011) and we found that copper tolerance of monocultures increased as a function of siderophore production across isolates (GLM: effect of siderophores on  $\Delta N$ :  $\chi_1^2 = 4.65$ ,  $p = 0.03$ ; Figure 4a). We, therefore, determined if siderophore production of a given isolate determined the extent to which it was affected by the presence of other species. This relationship differed in copper-polluted and unpolluted compost (LMM: copper  $\times$  siderophore production on  $d$ :  $\chi_1^2 = 24.46$ ,  $p < 0.001$ ; Figure 4b,c).

In unpolluted compost, there was no relationship between siderophore production and the sign of species interaction (estimated slope  $\pm 95\%$  CI:  $\beta_{\text{control}} = -34.5$  [ $-139, 70.2$ ]). This indicates that siderophore production, on average, is not an important predictor of interspecific interactions in unpolluted soil, suggesting species most likely competed for nutrients other than iron (Niehus et al., 2017). In the presence of copper, there was a negative relationship between the outcome of species interactions and siderophore production ( $\beta_{\text{copper}} \pm 95\%$  CI =  $-128$  [ $-233, -23.4$ ]) with isolates that benefitted most from co-culturing in copper (*Arthrobacter*, *Devosia* and *Flavobacterium*) producing the least amount of siderophore (Figure 1). Notably, *Flavobacterium* and *Devosia* were both negatively affected by competitors in unpolluted environments, but showed an absolute benefit from co-culturing under copper stress (Figure 4b,c). We found no evidence that an isolate's siderophore production affected the extent it facilitated the growth of its partner (LMM: effect of copper  $\times$  siderophore production on  $d$ :  $\chi_1^2 = 1.06$ ,  $p = 0.30$ ; main effect of siderophores:  $\chi_1^2 = 1.60$ ,  $p = 0.21$ ; Figure 3c), suggesting non-linear dose–response effects of siderophores within species.

## DISCUSSION

Consistent with recent *in vitro* work on bacteria isolated from tree holes (Foster & Bell, 2012) and metal working fluid (Piccardi et al., 2019), we found that interactions were largely negative under benign conditions in a natural compost environment. In line with the SGH (Bertness & Callaway, 1994; Bruno et al., 2003; He et al., 2013), we found that the addition of toxic copper stress induced a shift towards more positive interactions. This was primarily because some species were protected by others from the toxic effects of copper, but relaxed competition resulting from population size reductions may also have contributed. Our results are consistent with a role of



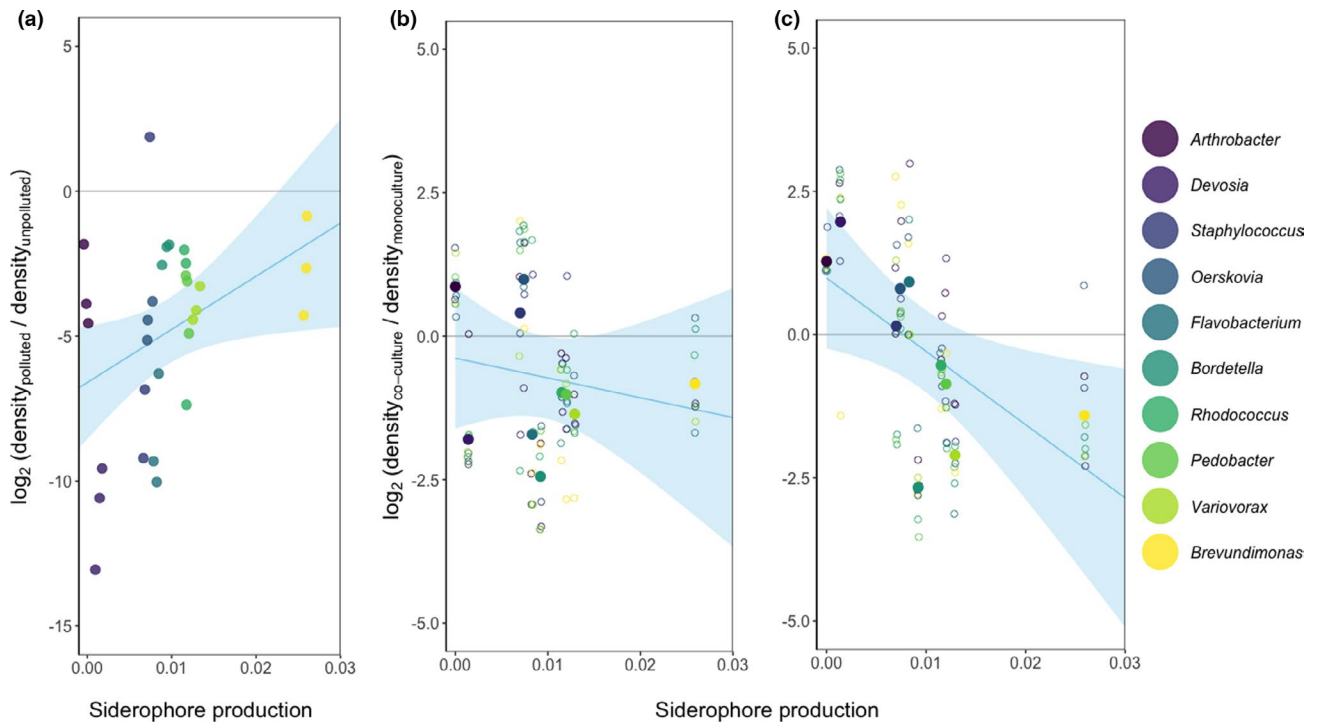
**FIGURE 3** Copper stress results in more positive species interactions. (a) Boxplots showing copper shifts the balance towards more positive interactions, where different colours depict the mean interaction sign for each isolate averaged across all possible pairwise competitions ( $n = 9$ ). (b, c) Linear networks depicting pairwise interactions in benign (b) and copper-polluted (c) conditions. Each arc connects two isolates. Arcs below the nodes run from right to left, and upper arcs run from left to right. In scenarios where  $d > 0$ , species benefitted from the presence of others (positive interactions, dark blue), whereas  $d < 0$  indicates isolates grew better in isolation (negative interaction, light blue). Arrow thickness is proportional to  $d$  (values of mean  $d > |1.25|$  are shown). Isolates were identified based on their distinct colony morphology, where A = *Arthrobacter*, C = *Devosia*, E = *Staphylococcus*, F = *Oerskovia*, J = *Flavobacterium*, I = *Bordetella*, G = *Rhodococcus*, H = *Pedobacter*, K = *Variovorax* and D = *Brevundimonas*. (d) Boxplots showing differences in relative performance during pairwise competition in copper-polluted versus unpolluted compost ( $\Delta d$ ). Positive values indicate a shift towards more facilitative interactions in response to copper stress, and negative values a shift towards more antagonistic interactions. For each focal isolate, boxplots are based on nine pairwise interactions, with the effect of different competitors on focal species performance depicted in different colours. Boxes depict the upper and lower quartiles of the treatment-specific data with the centre line showing the median and whiskers providing a measure of the 1.5 $\times$  interquartile range

siderophores in this protection (O'Brien et al., 2018), with siderophore producers 'rescuing' copper-sensitive taxa.

Given the correlational nature of our data, we cannot rule out the possibility that other detoxification traits could have played a role in ameliorating toxic environmental conditions. However, many traits that confer metal resistance largely benefit the detoxifier (O'Brien & Buckling, 2015), and not the wider community. For example, while intracellular sequestration renders the environment less toxic, this process likely operates over smaller spatial scales than siderophore-based detoxification: siderophores can diffuse into environment away

from the producer (Kummerli et al., 2014), and hence are more likely to benefit other species. Other detoxification traits, such as efflux pumps, are likely to negatively affect non-detoxifying neighbouring cells, and as such, cannot explain our finding of increased facilitation.

While we observed clear facilitation of species that produced relatively small amounts of siderophores, our previous work indicates that there is net selection against such taxa in natural microbial communities (Hesse et al., 2018). This suggests that the beneficial effects of siderophores are greatest for siderophore-producing cells compared with other cells growing in the near vicinity,



**FIGURE 4** The role of siderophores in the outcome of species interactions. (a) Plot depicting the linear relationship between copper-mediated changes in population density and siderophore production. Values of  $\Delta N < 0$  (grey horizontal line) indicate isolates performed worse in copper-polluted versus unpolluted conditions. Line and blue shaded area depict the fitted linear relationship  $\pm 95\%$  confidence intervals ( $\Delta N = -6.60[-8.55, -4.65] + 183.32[16.65, 349.98] \times \text{siderophores}$ ). (b, c) Abiotic stress changes the relationship between siderophore production and relative performance during pairwise competition, where small dots depict  $d$  for all possible pairwise competitions ( $n = 9$  per isolate) and large dots provide a measure of across-species mean performance in (b) unpolluted and (c) copper-polluted compost. Note that  $d$  in panels (b) and (c) is presented on an inverse hyperbolic sine scale. Line and shaded area depict the fitted relationships  $\pm 95\%$  CI (copper:  $\sinh^{-1}(d) = 0.99[-0.24, 2.22] - 128[-233, -23.4] \times \text{siderophores}$  and control:  $\sinh^{-1}(d) = -0.38[-1.61, 0.85] - 34.5[-139, 70.2] \times \text{siderophores}$ )

presumably because spatial structure limits the diffusion of siderophores (Kummerli et al., 2009). This localised benefit may also help to explain why benefits to one species were not accompanied by costs to the other during pairwise interactions. Assuming species were only partially rescued, they likely imposed less resource competition on the detoxifying species than they would have if they were completely rescued. Copper-mediated reductions in resource competition, in general, may also help to explain why isolates that promoted the growth of other species did not suffer a reciprocal negative impact.

The SGH has largely been studied in terrestrial plants and benthic marine invertebrates (Brooker et al., 2008; He et al., 2013), having received little attention in other life forms (but see: Barrio et al., 2013; Bowker et al., 2010; Fugère et al., 2012; Piccardi et al., 2019). While this hypothesis was originally developed to predict community-wide responses (Bertness & Callaway, 1994), predictions have largely been tested with one or very few pairwise interactions (He et al., 2013). Hence, there is no general consensus on the degree to which species interactions predictably change as a function of abiotic stress (Soliveres et al., 2015). Moreover, where there is support for enhanced facilitation in response to stress, the drivers and consequences of facilitation are often not understood (Michalet & Pugnaire, 2016). This is especially

relevant to bacterial communities, which have rarely been studied experimentally in the context of the SGH (Hernandez et al., 2021; Lawrence & Barraclough, 2015; Li et al., 2013; Piccardi et al., 2019; Rodríguez-Echeverría et al., 2016).

Our findings fill these knowledge gaps by demonstrating that (i) copper stress causes a shift towards net positive interactions in species that rely on others for habitat amelioration and (ii) the magnitude of, and cost associated with, facilitation is largely independent of a benefactor's siderophore production (see Schöb et al., 2014 for beneficiary feedback effects in terrestrial plants). Finally, our results highlight that secreted molecules that benefit other species ('community-wide public goods'), which also include antibiotic-degrading enzymes (Lee et al., 2010), virulence factors (Raymond et al., 2012) and nutrients (Gralka et al., 2020), may play an important role in shaping microbial community compositions. These compositional changes can, in turn, feedback to alter the evolution of community-wide public goods (Morris et al., 2012).

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## AUTHOR CONTRIBUTIONS

EH, AB, EvV and DH conceived and designed the experiment. EH, SOB, FB and AL collected the data. EH, DS and DH carried out data analyses. EH and AB wrote the first draft of the manuscript, and all authors contributed to revisions.

## PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/ele.13847>.

## DATA AVAILABILITY STATEMENT

The data supporting the results are archived in Zenodo: <https://doi.org/10.5072/zenodo.835531>.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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