Ecology, 96(8), 2015, pp. 2042–2048

Reports

Non-random species loss in bacterial communities reduces antifungal volatile production

W. H. Gera Hol,[1,4] Paolina Garbeva,[2] Cornelis Hordijk,[2] Maria P. J. Hundscheid,[2] Paulien J. A. Klein Gunnewiek,[2] Maaike van Agtmaal,[2] Eiko E. Kuramae,[2] and Wietse de Boer[2,3]

[1]*Netherlands Institute of Ecology, Department of Terrestrial Ecology, NIOO-KNAW, Droevendaalsesteeg 10, 6708 PB Wageningen, The Netherlands*
[2]*Netherlands Institute of Ecology, Department of Microbial Ecology, NIOO-KNAW, Droevendaalsesteeg 10, 6708 PB Wageningen, The Netherlands*
[3]*Wageningen University, Department of Soil Quality, Droevendaalsesteeg 4, 6708 PB Wageningen, The Netherlands*

Abstract. The contribution of low-abundance microbial species to soil ecosystems is easily overlooked because there is considerable overlap between metabolic abilities (functional redundancy) of dominant and subordinate microbial species. Here we studied how loss of less abundant soil bacteria affected the production of antifungal volatiles, an important factor in the natural control of soil-borne pathogenic fungi. We provide novel empirical evidence that the loss of soil bacterial species leads to a decline in the production of volatiles that suppress root pathogens. By using dilution-to-extinction for seven different soils we created bacterial communities with a decreasing number of species and grew them under carbon-limited conditions. Communities with high bacterial species richness produced volatiles that strongly reduced the hyphal growth of the pathogen *Fusarium oxysporum*. For most soil origins loss of bacterial species resulted in loss of antifungal volatile production. Analysis of the volatiles revealed that several known antifungal compounds were only produced in the more diverse bacterial communities. Our results suggest that less abundant bacterial species play an important role in antifungal volatile production by soil bacterial communities and, consequently, in the natural suppression of soil-borne pathogens.

Key words: antifungal volatiles; ecosystem functioning; functional redundancy; mass ratio hypothesis; microbial diversity; rare biosphere; removal experiment; species loss.

Introduction

Dominant species have long been considered to be responsible for most ecosystem processes (Grime 1998). However, several studies indicate that the role of rare species in these processes has been underestimated (Lyons and Schwartz 2001, Zavaleta and Hulvey 2004, Hol et al. 2010). The role of rare microbial species in soil is infrequently tested, even though recent advances in sequencing technology have revealed enormous numbers of rare species in the so-called "rare biosphere" (Pedros-Alio 2007). Bacterial communities in soil are extremely species-rich (Fierer and Jackson 2006) but there is considerable functional redundancy for primary metabolic processes (Mendes et al. 2015; e.g., the decomposition of organic compounds; Bell et al. 2005, Rousk et al. 2009). Consequently, the role of low-abundance microbial species in soil ecosystem functions related to decomposition, e.g., nutrient regulation, appears minor (Griffiths et al. 2001, Wertz et al. 2006, but see Baumann et al. 2013, Philippot et al. 2013). However, secondary metabolism by rare microbes may be a major contributor to other ecosystem functions such as biological control. So far, the role of low-abundance microbial species in processes based on secondary metabolism, such as symbiosis, resistance, iron acquisition or production of antimicrobial compounds has hardly been addressed.

Suppression of disease caused by soil-borne pathogens is a wide-spread phenomenon (Garbeva et al. 2011*a*) that contributes worldwide to natural control of fungal diseases in crops. Several bacterial species that produce antimicrobial secondary metabolites (Mendes et al. 2011) have been suggested to play a prominent role in disease suppression. Among the bacterial secondary metabolites that inhibit plant-pathogenic fungi, increasing attention is being paid to volatiles. It is estimated that one-third of all cultivable soil bacteria species are able to produce antifungal volatiles (Effmert et al. 2012). Several of these volatiles have also been detected in the belowground atmosphere (Zou et al. 2007). Microbial volatiles, due to their ability to travel through air and water, can play two major roles in long-distance interactions in microbial communities as (1) infochemical molecules affecting the behavior, population dynamics, and gene expression in the responding microorganism (Garbeva et al. 2014*a*) and (2) competitive tools directly exerting antagonistic activity, providing an ecological advantage by suppressing or eliminating potential enemies (Insam and Seewald

Manuscript received 8 December 2014; revised 23 March 2015; accepted 1 April 2015. Corresponding Editor: S. D. Allison.

[4] E-mail: g.hol@nioo.knaw.nl

2010, Effmert et al. 2012, Garbeva et al. 2014*b*). However, volatile production by soil bacterial species is mostly examined using monocultures of bacteria and nutrient-rich media. This is in strong contrast with the conditions in soil where microbial species have to compete with each other for the limited organic energy resources (Demoling et al. 2007), and it is unknown how this would affect the production of antifungal volatiles.

Production of volatiles is expected to be largely determined by the more abundant species, as they are successful competitors that metabolize most of the available organic compounds. However, the amount and composition of secondary metabolites produced by bacterial species is affected by the identity of competing species (Garbeva et al. 2011*b*) and species-rich bacterial communities show more antagonistic interactions (Becker et al. 2012). Thus, high bacterial diversity may trigger the production of a higher diversity of volatile compounds (Jousset et al. 2014). In addition, suppression of fungi by volatiles may be due to mixtures of compounds rather than to single compounds (Strobel et al. 2001, Tunc et al. 2007, Veras et al. 2012). Therefore, we tested the hypothesis that loss of less abundant bacterial species decreases antifungal volatile production, resulting in increased growth of fungal pathogens. Our definition of rarity is based on local abundance in cultivated bacterial communities, and we consider the abundance of rare species to be less than 1% of the total abundance in the least diluted community.

We examined the effect of soil bacterial species loss on the suppression of root pathogenic fungi with a dilution-to-extinction series. Soil bacterial communities were diluted and cultivated on water yeast agar (Salonius 1981, Franklin and Mills 2006), a carbon-poor medium, to simulate soil-like conditions with respect to limited availability of energy resources (De Boer et al. 2007). Dilution-to-extinction series have the advantage that diluted communities consist of naturally co-occurring species and generally cover a wider diversity range than most biodiversity-functioning studies using community assembly. Therefore, by using the non-random species loss by dilution method on a range of soils we were able to test how the loss of less abundant bacteria species affects the production of antifungal volatiles and consequently fungal growth.

Methods

Description of sampling sites

Soil samples were collected from several agricultural and ex-agricultural sites in the Netherlands in August 2009 (Appendix: Table A1). Soils were passed through a sieve (4 mm) and stored at 4°C until further use. Part of the soil was dried at 40°C for determining available nutrients. Plant available phosphorus (Olsen P) and soil pH-H_2O were measured as described by Troelstra et al. (1995). The percentages carbon and nitrogen in the soil were determined on an elemental autoanalyzer (Flash EA 1112 NC Interscience, Breda, the Netherlands).

Dilution experiment with multiple pathogens.—The effect of serial dilution of soil bacterial inocula from soil A (Appendix: Table A1) was tested on the growth of three plant pathogenic fungal species, *Fusarium culmorum*, *Fusarium oxysporum*, and *Rhizoctonia solani* and one plant pathogenic oomycete, *Pythium intermedium* (for origin of strains see Appendix: Table A2). A suspension of 10 g dry mass soil and 50 mL phosphate buffer (KH_2PO_4, 1 g/L) pH 6.5 was mixed on a rotary shaker for 1.5 h, followed by 1 minute sonification at 47kHz twice and shaking for 0.5 h again (Garbeva et al. 2014*a*). Suspensions were plated on carbon-limited water yeast agar plates (composition: 1 g/L KH_2PO_4, 0.1 g/L $(NH_4)_2SO_4$, 0.1 g/L yeast extract (Bacto), 20 g/L CMN agar (Boom); pH 6.5) including filter-sterilized fungal inhibitors 100 mg/L cycloheximide (Sigma-Aldrich, St. Louis, Missouri, USA) and 50 mg/L thiabendazole (Sigma-Aldrich). In another experiment, those concentrations were found to be effective inhibitors of fungal growth. Three subsamples of soil were used to create three independent suspensions as replicates. For four dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}) 50 μL of this suspension was plated on carbon-limited water yeast agar plates. Bacteria were grown at 20°C for one week, after which the bacterial biomass was spread over the entire agar surface by adding glass beads and shaking the plates. Glass beads were removed and all plates were then incubated again at 20°C for one week. The aim of this spreading was to obtain similar biomasses on plates inoculated with different dilutions (see Appendix for discussion).

Next, pathogenic fungi were introduced on agar-containing lids and fungi were thus exposed to the volatiles produced by the bacteria but without direct contact between bacteria and pathogen. For this fungal inhibition test we used an inverted assay (Garbeva et al. 2014*a*; Appendix: Fig. A1) with bacteria growing on the bottom of the Petri dish and fungi on the lid. The growth of three fungal (*F. culmorum*, *F. oxysporum*, *R. solani*) and one oomycete (*P. intermedium*) species was measured as hyphal extension. Inhibition of fungal growth on the lids is calculated as the percentage of extension of hyphae on control lids exposed to bacteria-free water-yeast agar as $100 \times (1 - [G_p/G_a])$, where G_p is growth in the presence of bacteria and G_a is growth in the absence of bacteria.

Dilution experiment with seven soils.—Three replicate suspensions were made for each of the seven soils (Appendix: Table A1). Four dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}) of each suspension were used to test the inhibition of *F. oxysporum* growth by bacterial volatiles. Bacterial biomass was collected by washing the plates three times with 5 mL sterile water and carefully scraping the bacteria from the agar surface. The procedure was repeated for agar plates without bacteria as a background control. The collected 15 mL was divided as follows: 1 mL stored at −20°C for DNA extraction, 1 mL at −80°C as backup and the remaining 13 mL was freeze dried, weighed, and the percentage of carbon was determined (soils A–D) on an elemental auto analyzer Flash EA 1112 NC (Interscience, Breda, the Netherlands). Three replicates were measured for each of the dilution

treatments. Total carbon was calculated and used as estimate of bacterial biomass per dilution treatment.

Dilution experiment including volatile-absorbing, activated coal.—Three independent soil suspensions were made for each of the seven soils (Appendix Table A1) and 10^{-1} dilutions were plated out as described before. After 2 weeks incubation for the bacteria, the growth of *F. oxysporum* on water yeast agar was tested in absence or presence of activated charcoal. In the agar, a circle was punched out, in which a stainless steel ring (height 12 mm and ø 16 mm) was placed, filled with 1 g activated charcoal 10–18 (Fisher Scientific, Landsmeer, The Netherlands). Areal extension of *F. oxysporum* colonies was determined and inhibition was calculated as described before.

Isolation of DNA, amplification, and pyrosequencing of 16S rRNA genes

Characterization of the bacterial communities at the end of the dilution experiment with seven soils was based on the collected bacterial cell suspensions stored at −20°C. All three replicates of each dilution treatment were analyzed for soils A–D and F, while for soils E and G one or two replicates were done. DNA was extracted from the bacterial suspensions and used as a template in a PCR reaction with the primer set 515F and 806R targeting the V4 region of the bacterial 16S rRNA gene (Caporaso et al. 2011). More information on thermocycling conditions and purification of samples can be found in the Appendix. The samples were sequenced on a Roche 454 automated sequencer (454 Life Sciences, Branford, Connecticut, USA) and GS FLX system using titanium chemistry (Macrogen, Seoul, South Korea). Pyrosequencing data-processing is described in the Appendix. OTUs (operational taxonomy units) were defined at the 97% sequence similarity level. In total, 422 120 high-quality sequences were obtained, with reads per sample ranging from 1045 to 10 525. Relative abundance of different bacterial groups was estimated in each bacterial community by comparing the number of sequences classified as belonging to the specific bacterial groups to the total number of classified bacterial sequences per sample.

Volatiles trapping and analysis of bacterial volatiles

For the collection of bacterial volatiles Petri-dishes glass lids were used with a "chimney" to which a steel trap with 150 mg Tenax TA and 150 mg Carbopack B (Markes International, Llantrisant, UK) could be fixed (Garbeva et al. 2014*a*). Collection of volatile compounds was performed for dilution treatments 10^{-1} and 10^{-3} for two soils (D and F) that showed a clear effect of dilution treatment. There were five independent replicates for each soil type. Sterile Petri dishes of water-yeast agar served as background controls. Volatiles were collected over 10 days. Traps were removed, capped, and stored at 4°C until analysis on a gas chromatograph combined with a mass spectrometer (GC-MS; Appendix). The linear retention indexes (lri) values were compared with those found in the NIST and the NIOO lri database. Peaks detected in the controls (340 peaks in total) were removed from the data set. Candidate compounds that might cause fungal volatile inhibition were identified by screening for volatiles that were more abundant in bacterial communities developed from the 10^{-1} dilution treatment than in bacterial communities developed from the 10^{-3} dilution treatment. For an overall representation of volatile profiles across treatments an NMDS plot was made based on Bray-Curtis dissimilarity, using the R packages vegan and labdsv (Appendix: Fig. A11).

Statistical analyses

For both the four-pathogen assay and seven soil assay we used ANOVA to test the main and interactive effects of the dilution treatment and species on inhibition. Both data sets were rank transformed because the residuals were not normally distributed (Kolmogorov-Smirnov test $P < 0.001$). Planned comparisons between dilution treatments 1 vs. 2, 2 vs. 3, and 3 vs. 4 were done with Wilcoxon paired tests. Differences in bacterial biomass between dilution treatments were tested with ANOVA on ranked data, with the interaction between dilution treatment and soil origin as explanatory variables. Pyrosequencing data were rarefied to 1000 reads per sample. The average number of OTUs per soil and dilution treatment were calculated and tested non-parametrically with the Friedman test, using soil as block, followed by pairwise comparisons with Wilcoxon tests. The correlations between OTU richness and fungal inhibition were analyzed with Spearman rank correlation tests. All statistical analyses were done with the base package in R 3.0.0 (R Core Team 2013).

Results

An initial study showed that the hyphal growth of four pathogens was significantly inhibited when exposed to volatiles produced by soil bacterial communities developing on carbon-limited agar (Appendix: Fig. A2). Based on this study, *F. oxysporum* was selected to investigate suppressing volatile production by different soil bacterial communities from seven different soil origins (Appendix: Table A1). All these communities produced volatiles that reduced the growth of *F. oxysporum* (Figs. 1 and A3). The presence of volatile-absorbing activated coal in the plates decreased inhibition of fungal growth by 50% (Appendix: Fig. A4). The inhibition of *F. oxysporum* by volatiles was also observed when the fungus was exposed to each of the soils from which the bacteria were originally extracted (Appendix: Fig. A5). Serial dilution of the inoculated soil bacterial communities led to an overall significant decline in inhibition of *F. oxysporum* (Fig. 1), which could not be ascribed to a dilution treatment effect on bacterial biomass (Appendix: Fig. A6). Only for the strongest dilution treatment did bacterial biomass tend to be lower in comparison to the weakest dilution treatment. The effect of dilution remained significant even when biomass was included in the statistical

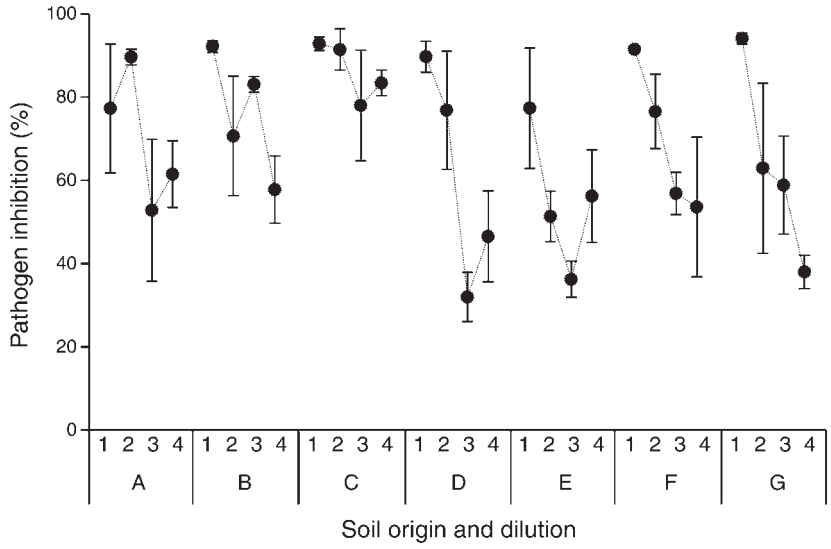

FIG. 1. Inhibition of the fungal root pathogen *Fusarium oxysporum* by volatiles from soil bacterial communities developing on nutrient-poor agar. Values are the mean (±SE) inhibition of *Fusarium oxysporum* exposed to bacterial volatiles decreases with dilution of inoculated soil bacterial communities (based on dilution series [where 1 is 10^{-1} dilution, 2 is 10^{-2} dilution, 3 is 10^{-3} dilution, and 4 is 10^{-4} dilution] of suspensions from seven soils [A–G], $n = 3$ per dilution per soil). Soil origin df = 6, 14, $F = 3.377$, $P = 0.028$; dilution df = 1, 14, $F = 31.98$, $P < 0.001$; soil origin × dilution df = 6, 14, $F = 0.893$, $P = 0.526$. Dilution treatments 1–2 and 2–3 are significantly different (Wilcoxon $V = 26$, $n = 7$, $P = 0.047$); dilution treatments 3 and 4 are not ($V = 14$, $n = 7$, $P = 1$).

analyses as covariable (dilution df = 1, 38, $F = 15.77$, $P < 0.001$) despite the significant effect of biomass on inhibition (biomass df = 1, 38, $F = 4.70$, $P = 0.036$).

Analysis of sequence data revealed that the diversity of all bacterial communities declined with dilution treatment at all taxonomic levels, from genera to phyla (Figs. 2b and 3a, Appendix: Table A3). Serial dilution reduced the number of rare species (Appendix: Fig. A7) and their cumulative abundance (Appendix: Fig. A8). In dilution treatment 2, 44% ± 4% of the species with relative abundance <1% in the least diluted treatment were not detected; in dilution treatment 3, this was 62% ± 4%, and in dilution treatment 4, 84% ± 3% (means ± SE, $n = 6$–7). For three communities (B, D, F) this diversity decline coincided with a significant decrease in volatile inhibition of *F. oxysporum* and more communities showed the same trend (Fig. 3b, Appendix: Fig. A9). At the lowest dilution treatment (i.e., highest species diversity) the reduction in fungal growth was rather consistent across all soil origins with volatile inhibition ranging from 77% to 94% compared to the control without bacteria. The effect of species loss on volatile suppression of fungi varied between different soil bacterial communities (Appendix: Fig. A9): dilution of some communities (D and F) resulted in drastic reductions in fungal inhibition and others (C) maintained the inhibition at continuously high levels. The composition of the bacterial communities varied strongly (Fig. 2a, Appendix: Fig. A10), probably due to differences in soil properties and land use history (Appendix: Table A1).

Multiple bacterial taxa seem to be involved in the production of antifungal volatiles as inhibition of *F. oxysporum* occurred with strongly different soil bacterial communities (Fig. 1 and Fig. 2a). There is only a weak correlation between overall bacterial community composition and the level of fungal inhibition (Appendix: Fig. A10). This hampers identification of key volatile-producing bacterial taxa. At genus level, there were several groups that occurred only (or more frequently) in the lowest diluted communities that caused high level of fungal inhibition (Appendix: Table A3). These low-abundance genera appear to play an important role in the production of antifungal volatiles. Analyses of volatile compounds were performed for dilutions 1 and 3 of the two soil origins (D and F) that showed the most clear decrease of fungal suppression with increasing dilution treatment (Appendix: Fig. A9). The total number of volatile peaks was not significantly different between the two dilutions treatments. Comparing dissimilarity in volatile GC-MS profiles from different dilution treatments with NMDS showed that the two soil origins had very distinctive volatiles patterns that separated on the first NMDS axis; and within soil origin the dilution treatments resulted in shifts in volatile profiles that separated on the second NMDS axis (Appendix: Fig. A11). Volatiles that occurred in all replicates of the least diluted treatment and less frequently in the high dilution treatment included the antifungal compounds 2-methylfuran, 2-furaldehyde, 2-(methylthio)benzothiazole, and muurolol (Appendix: Table A4). The candidate volatiles that might cause fungal suppression differed between the two soil origins (Appendix: Table A4), which is in line with their different bacterial community compositions.

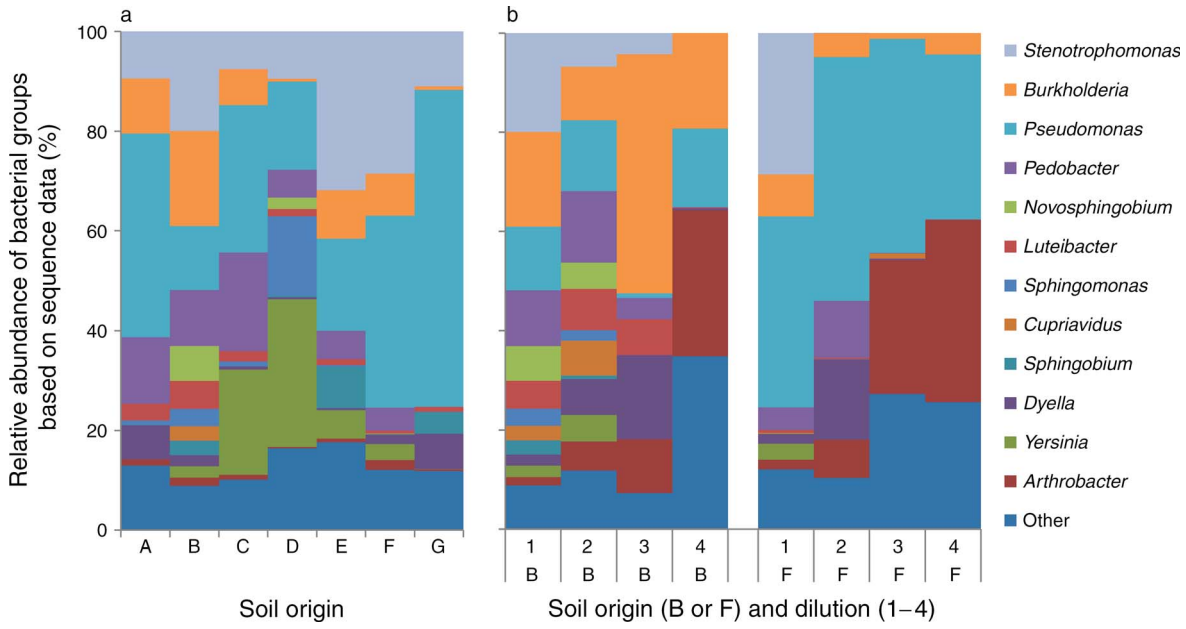

FIG. 2. Relative abundance of the most dominant bacterial genera growing on nutrient-poor agar inoculated with serial dilutions of soil suspensions (a) in the least-diluted inoculum of seven soils (A–G) listed in Appendix: Table A1 and (b) in the complete dilution series of two soils (B and F) as examples. The numbers 1–4 indicate levels of 10-fold dilution, with 1 least diluted and 4 most diluted.

DISCUSSION

Our results indicate that diverse bacterial communities play a thus far underestimated role in the suppression of plant-pathogenic fungi and, consequently, in plant health. The antifungal effect of diverse bacterial communities could be due to species-specific volatile production or due to species interactions. Since the species lost after dilution generally have low abundance, the amount of volatiles they produce is also expected to be low. This implies that direct production by low-abundance species can only contribute to fungal suppression when these volatiles have a relatively high toxicity, when many low-abundance species produce the same compound or when the combination with volatiles produced by dominant species results in stronger inhibition of the fungus, for example by synergistic effects (Tunc et al. 2007, Veras et al. 2012). It is certainly possible for species-specific effects to occur in all soils and that low-abundance taxa from a wide number of phyla are producing antifungal volatiles. However, given the rather consistent effects of species loss from different soil bacterial communities, the most parsimonious explanation for the decline in antifungal volatiles with dilution treatment seems to be the decline in species interactions. There are several examples of interactions between bacterial strains affecting secondary metabolite production (Garbeva et al. 2011*b*, 2014*b*, Becker et al. 2012, Jousset et al. 2014), and the results of our study lead to the hypothesis that low-abundance bacterial species may trigger volatile production in dominant species. The alternative hypothesis being that low-abundance bacterial species produce antifungal volatiles themselves.

One aspect of dilution-to-extinction is that the loss of rare species is confounded with overall diversity. Additional experiments with assembled communities would be required to see whether diversity loss in general leads to reduced antifungal volatile production, or only when low-abundance species disappear. The large variation in the level of suppression (30–89%) at low diversity (4–16 OTUs) suggests that both diversity and community composition are important for volatile suppression. Identification of bacterial taxa responsible for the production of these antifungal volatiles is difficult because the volatile profile of candidate taxa in monoculture would not necessarily show their metabolic capabilities in a community context.

Apart from suggesting a novel role of subordinate species in the natural control of root-infecting pathogens, our results elucidate an important issue in the ongoing debate regarding species gains and losses (Wardle et al. 2011): losses of subordinate species can have consequences for ecosystem functions and should be included when considering consequences of biodiversity loss and gain. Species with low abundances are at higher risk of local extinction (Gaston 2008) and here we showed how the loss of rare bacterial species can reduce pathogen suppression, an ecosystem service of soils. An important next step will be to test whether low-abundance bacteria are also involved in the suppression of human pathogens that have a soil-borne stage (Fisher et al. 2012). Bacterial species loss has been demonstrated to favor invasion of a soil-borne bacterial pathogen due to reduced resource competition (van Elsas et al. 2012, Mallon et al. 2015).

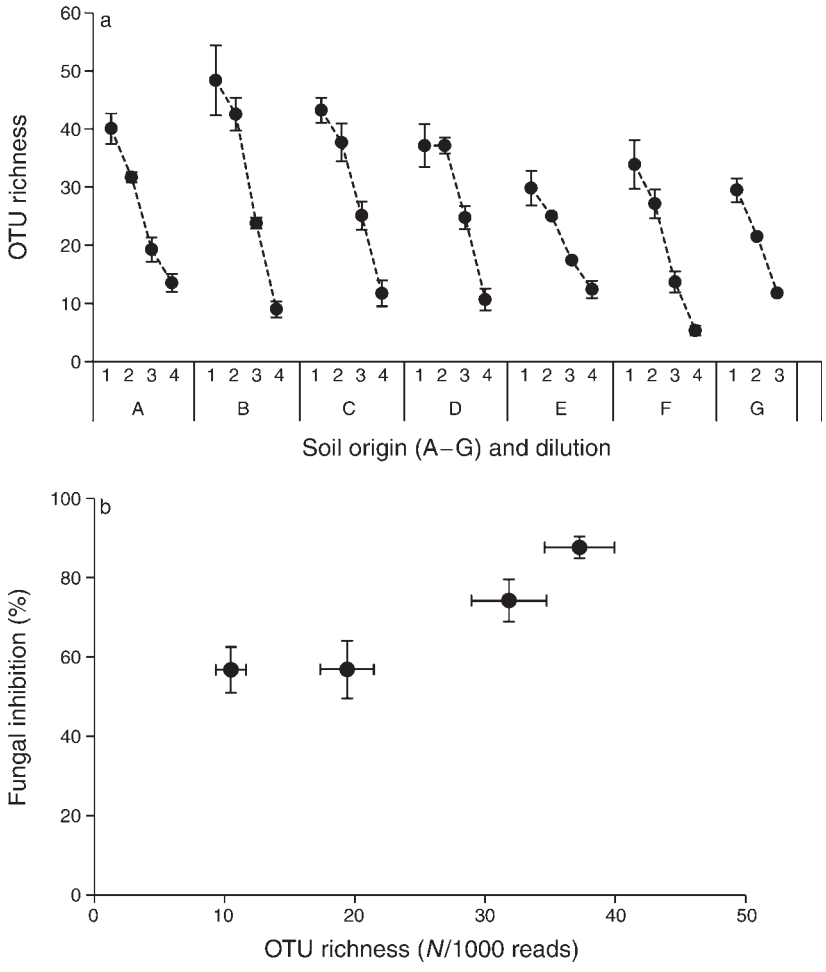

FIG. 3. (a) Operational taxonomic units (OTU) richness in seven cultivated soil bacterial communities on nutrient-poor agar decreases with increasing dilution. Values are means ± SE, $n = 3$ except $n = 1$–2 for soil origins E and G. Soil origins are A–G in Appendix: Table A1; 1–4 indicates level of 10-fold dilution, with 1 least diluted and 4 most diluted. All dilution treatments are significantly different from each other across all soil origins $P < 0.05$. Soil origin df = 6, 14, $F = 3.334$, $P = 0.033$; dilution df = 1, 14, $F = 26.068$, $P < 0.001$; soil origin × dilution df = 6, 14, $F = 0.776$, $P = 0.60$). (b) Inhibition of growth of the plant-pathogenic fungus *F. oxysporum* by volatiles produced by dilution series of soil bacterial communities developing on nutrient-poor agar decreases with OTU loss. Values are means ± SE and are averaged per dilution treatment across all seven soil origins ($n = 3$). (Spearman $S = 1136$, $\rho = 0.65$, $n = 28$, $P < 0.05$). Data for each soil origin are shown in Appendix: Fig. A9.

Here we provide an additional mechanism by which diverse communities can suppress pathogens without direct contact. This functional aspect of soil microbial communities has important consequences for our understanding of soil health. Reductions in species abundance after soil disturbance might already drastically change ecosystems (Fierer et al. 2013) and with this study a possible mechanism behind such shifts has been revealed.

ACKNOWLEDGMENTS

We thank Mattias de Hollander for help with the bioinformatics analyses. Gera van Os and Marjan de Boer from Applied Plant Research provided the *Fusarium* and *Pythium* strains. We thank Wim van der Putten and Kelly Ramirez for comments and advice. W. Hol is financially supported by the Centre for Soil Ecology (NIOO-WUR). This is publication 5837 of the NIOO-KNAW.

LITERATURE CITED

Baumann, K., M. F. Dignac, C. Rumpel, G. Bardoux, A. Sarr, M. Steffens, and P. Maron. 2013. Soil microbial diversity affects soil organic matter decomposition in a silty grassland soil. Biogeochemistry 114:201–212.

Becker, J., N. Eisenhauer, S. Scheu, and A. Jousset. 2012. Increasing antagonistic interactions cause bacterial communities to collapse at high diversity. Ecology Letters 15:468–474.

Bell, T., J. A. Newman, B. W. Silverman, S. L. Turner, and A. K. Lilley. 2005. The contribution of species richness and composition to bacterial services. Nature 436:1157–1160.

Caporaso, J. G., C. L. Lauber, W. A. Walter, D. Berg-Lyons, C. A. Lozupone, P. J. Turnbaugh, et al. 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Sciences USA 108:4516–4522.

De Boer, W., A. M. Wagenaar, P. J. Klein Gunnewiek, and J. A. Van Veen. 2007. In vitro suppression of fungi caused by combinations of apparently non-antagonistic soil bacteria. FEMS Microbiology Ecology 59:177–185.

Demoling, F., D. Figuerora, and E. Bååth. 2007. Comparison of factors limiting bacterial growth in different soils. Soil Biology and Biochemistry 39:2485–2495.
Effmert, U., J. Kalderás, R. Warnke, and B. Piechulla. 2012. Volatile mediated interactions between bacteria and fungi in the soil. Journal of Chemical Ecology 38:665–703.
Fierer, N., and R. B. Jackson. 2006. The diversity and biogeography of soil bacterial communities. Proceedings of the National Academy of Sciences USA 103:626–631.
Fierer, N., et al. 2013. Reconstructing the microbial diversity and function of pre-agricultural tallgrass prairie soils in the United States. Science 342:621–624.
Fisher, M. C., et al. 2012. Emerging fungal threats to animal, plant and ecosystem health. Nature 484:186–194.
Franklin, R. B., and A. L. Mills. 2006. Structural and functional responses of a sewage microbial community to dilution-induced reductions in diversity. Microbial Ecology 52:280–288.
Garbeva, P., W. H. G. Hol, A. J. Termorshuizen, G. A. Kowalchuk, and W. de Boer. 2011*a*. Fungistasis and general soil biostasis—a new synthesis. Soil Biology and Biochemistry 43:469–477.
Garbeva, P., C. Hordijk, S. Gerards, and W. de Boer. 2014*a*. Volatiles produced by the mycophagous soil bacterium *Collimonas*. FEMS Microbiology Ecology 87:639–649.
Garbeva, P., C. Hordijk, S. Gerards, and W. de Boer. 2014*b*. Volatile-mediated interactions between phylogenetically different soil bacteria. Frontiers in Microbiology 5:artikel 5.
Garbeva, P., M. W. Silby, J. M. Raaijmakers, S. B. Levy, and W. de Boer. 2011*b*. Transcriptional and antagonistic responses of *Pseudomonas fluorescens* Pf0-1 to phylogenetically different bacterial competitors. ISME Journal 5:973–985.
Gaston, K. J. 2008. Biodiversity and extinction: the importance of being common. Progress in Physical Geography 32:73–79.
Griffiths, B. S., et al. 2001. An examination of the biodiversity-ecosystem function relationship in arable soil microbial communities. Soil Biology and Biochemistry 33:1713–1722.
Grime, J. P. 1998. Benefits of plant diversity to ecosystems: immediate, filter and founder effects. Journal of Ecology 86: 902–910.
Hol, W. H. G., et al. 2010. Reduction of rare soil microbes modifies plant–herbivores interactions. Ecology Letters 13: 292–301.
Insam, H., and M. S. A. Seewald. 2010. Volatile organic compounds (VOCs) in soil. Biology and Fertility of Soils 46: 199–213.
Jousset, A., J. Becker, S. Chatterjee, P. Karlovsky, S. Scheu, and N. Eisenhauer. 2014. Biodiversity and species identity shape the antifungal activity of bacterial communities. Ecology 95:358–365.
Lyons, K. G., and M. W. Schwartz. 2001. Rare species loss alters ecosystem function–invasion resistance. Ecology Letters 4:358–365.
Mallon, C. A., F. Poly, X. Le Roux, I. Marring, J. D. van Elsas, and J. F. Salles. 2015. Resource pulses can alleviate the biodiversity-invasion relationship in soil microbial communities. Ecology 96:915–926.
Mendes, L. W., S. M. Tsai, A. A. Navarrete, M. de Hollander, J. van Veen, and E. E. Kuramae. 2015. Soil-borne microbiome: linking diversity to function. Microbial Ecology. http://dx.doi.org/10.1007/s00248-014-0559-2
Mendes, R., et al. 2011. Deciphering the rhizosphere microbiome for disease-suppressive bacteria. Science 332:1097–1100.
Pedros-Alio, C. 2007. Dipping into the rare biosphere. Science 315:192–193.
Philippot, L., et al. 2013. Loss in microbial diversity affects nitrogen cycling in soil. ISME Journal 7:1609–1619.
R Development Core Team. 2013. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/
Rousk, J., P. C. Brookes, and E. Baath. 2009. Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. Applied Environmental Microbiology 75:1589–1596.
Salonius, P. O. 1981. Metabolic capabilities of forest soil microbial-populations with reduced species-diversity. Soil Biology and Biochemistry 13:1–10.
Strobel, G. A., E. Dirkse, J. Sears, and C. Markworth. 2001. Volatile antimicrobials from *Muscodor albus*, a novel endophytic fungus. Microbiology 147:2943–2950.
Troelstra, S. R., R. Wagenaar, W. Smant, and W. de Boer. 1995. Soil-nitrogen transformation and nitrate utilization by *Deschampsia flexuosa* (L.) Trin. at 2 contrasting heathland sites. Plant Soil 176:81–93.
Tunc, S., E. Chollet, P. Chalier, L. Preziosi-Belloy, and N. Gontard. 2007. Combined effect of volatile antimicrobial agents on the growth of *Penicillium notatum*. International Journal of Food Microbiology 113:263–270.
van Elsas, J. D., M. Chiurazzi, C. A. Mallon, D. Elhottova, V. Kristufek, and J. F. Salles. 2012. Microbial diversity determines the invasion of soil by a bacterial pathogen. Proceedings of the National Academy of Sciences USA 109:1159–1164.
Veras, H. N. H., et al. 2012. Synergistic antibiotic activity of volatile compounds from the essential oil of *Lippia sidoides* and thymol. Fitoterapia 83:508–512.
Wardle, D. A., R. D. Bardgett, R. M. Callaway, and W. H. van der Putten. 2011. Terrestrial ecosystem responses to species gains and losses. Science 332:1272–1277.
Wertz, S., et al. 2006. Maintenance of soil functioning following erosion of microbial diversity. Environmental Microbiology 8:2162–2169.
Zavaleta, E. S., and K. B. Hulvey. 2004. Realistic species losses disproportionately reduce grassland resistance to biological invaders. Science 306:1175–1177.
Zou, C. S., M. H. Mo, Y. Q. Gu, J. P. Zhou, and K. Q. Zhang. 2007. Possible contributions of volatile-producing bacteria to soil fungistasis. Soil Biology and Biochemistry 39:2371–2379.

Supplemental Material

Ecological Archives

The Appendix is available online: http://dx.doi.org/10.1890/14-2359.1.sm

Data Availability

Data have been archived in the Data and Information Portal of the NIOO-KNAW: http://data.nioo.knaw.nl/index.php

Raw 454-pyrosequencing data have been deposited to the EMBL database (European Nucleotide Archive): http://www.ebi.ac.uk/ena/data/view/PRJEB5295