Soil biota and exotic plant invasion

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Invasive plants are an economic problem and a threat to the conservation of natural systems. Escape from natural enemies might contribute to successful invasion[1], with most work emphasizing the role of insect herbivores[2–4]; however, microbial pathogens are attracting increased attention[5]. Soil biota in some invaded ecosystems may promote 'exotic' invasion[6–9], and plant–soil feedback processes are also important. Thus, relatively rare species native to North America consistently demonstrate negative feedbacks with soil microbes that promote biological diversity[10], whereas abundant exotic and native species demonstrate positive feedbacks that reduce biological diversity[10]. Here we report that soil microbes from the home range of the invasive exotic plant *Centaurea maculosa* L. have stronger inhibitory effects on its growth than soil microbes from where the weed has invaded in North America. *Centaurea* and soil microbes participate in different plant–soil feedback processes at home compared with outside *Centaurea*'s home range. In native European soils, *Centaurea* cultivates soil biota with increasingly negative effects on the weed's growth, possibly leading to its control. But in soils from North America, *Centaurea* cultivates soil biota with increasingly positive effects on itself, which may contribute to the success of this exotic species in North America.

Soil microbes have profound negative and beneficial effects on plants through pathogenic effects, root–fungus mutualisms and by driving the nutrient cycles on which plants depend[5,11–17]. These effects, and the reciprocal effects of plants on soil microbes, contribute to two contrasting dynamic feedback interactions between plants and the microbial communities that develop around their roots[18,19]. Positive feedbacks occur when plant species accumulate microbes near their roots that have beneficial effects on the plants that cultivate them, such as mycorrhizal fungi and nitrogen fixers. Positive feedbacks are thought to lead to a loss of local community diversity[18,19]. Negative feedbacks occur when plant species accumulate pathogenic microbes in their rhizospheres, creating conditions that are increasingly hostile to the plants that cultivate the pathogens[10,20,21]. Negative feedbacks are thought to enhance community diversity by increasing species turnover rates.

Here we show that the direct effects of soil biota and the feedback loops that develop between soil biota and an invasive plant depend on the biogeographical source of the microbes. We compared the effects on *C. maculosa* growth of soil microbial communities collected from four populations of *C. maculosa* in its native range in western Europe to the effects of soil microbes collected from six populations in the northwestern United States where *C. maculosa* has invaded. On average, sterilization of European soils caused a 166% increase in the total biomass of *C. maculosa* compared with a 24% increase when North American soils were sterilized (Fig. 1). Soil sterilization can cause nutrient flushes; however, all pots were well fertilized and the effects of sterilization were consistent for multiple *C. maculosa* populations sampled over wide areas in Europe and North America, suggesting that the sterilization effect is not simply a peculiarity of a given site. However, the effect of soil sterilization varied among populations within continents. Depending on the *C. maculosa* population from which the soil microbial community was sampled and whether or not soil was sampled from *C. maculosa* or grass rhizospheres, sterilization of European soils improved *C. maculosa* growth from as little as 31% to over 900%, whereas the effects of sterilizing North American soils ranged from a 24% decrease in *C. maculosa* growth (suggesting a positive effect of microbes) to a 148% increase (Supplementary Information). The stronger suppressive effects of European soil biota lend experimental support to earlier demonstrations of much higher fungal and viral infection on plant species in their home ranges than in invaded ranges[5], and indicate that *C. maculosa* in North America have escaped the controlling effects of soil biota.

We further examined biogeographical differences in plant–soil microbe relationships by using soils from the *C. maculosa* population in the Central Massif in France and soils from Missoula, Montana, in a feedback experiment. We chose these because they represented the extreme cases: strong positive sterilization effects (inhibitory soil biota) on soils from the native range in the Central Massif, and weak negative sterilization effects (beneficial soil biota) on soils from the invaded range in Missoula (Supplementary Fig. 1). The microbial community in the soil from France was 'pre-cultured'[18–21] by planting either *C. maculosa* or *Festuca ovina*, a small perennial bunchgrass that is native to Eurasia, in pots with the original soils and allowing the plants to interact with the soil community for 3 months. The microbial community in the soil from Montana was pre-cultured by planting *C. maculosa* or *Festuca idahoensis*, a bunchgrass similar to *F. ovina* but native to western North America, in pots with the original soils. The pre-cultured soils with their microbial communities were then used to inoculate media in which we grew *C. maculosa* alone or in competition with one of the two grass species.

As observed in the first experiment, *C. maculosa* plants grown in Montana soils were much larger than those grown in French soils when soils were not sterilized. However, in this experiment substantial feedback loops between soil microbes and *C. maculosa* were demonstrated (Fig. 2). *Centaurea maculosa* plants grown alone in non-sterile French soil pre-cultured by conspecifics were significantly smaller than those grown in French soils pre-cultured by *F. ovina*. In contrast, *C. maculosa* planted alone in Montana soils

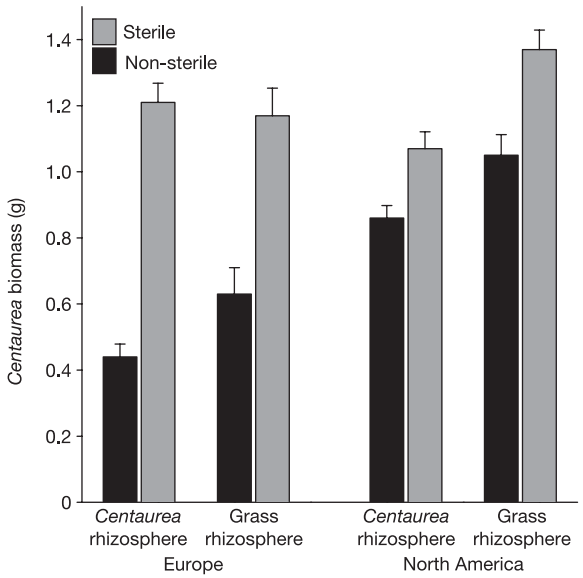

Figure 1 Total biomass of *C. maculosa* plants grown in non-sterilized and sterilized soil collected from European ($n = 4$) and North American ($n = 6$) populations of *C. maculosa*. Soils were collected from the rhizospheres of neighbouring grasses and from the rhizospheres of *C. maculosa*. In a 4-way analysis of variance (ANOVA) (region of origin, rhizosphere and sterilization as main effects, and population nested within region of origin) $F_{origin} = 40.18$, degrees of freedom (d.f.) = 1,297, $P < 0.001$; $F_{pop} = 21.81$, d.f. = 5,297, $P < 0.001$; $F_{rhiz} = 21.12$, d.f. = 1,297, $P < 0.001$; $F_{sterilization} = 110.87$, d.f. = 1,297, $P < 0.001$; $F_{origin \times sterilization} = 29.35$, d.f. = 1,297, $P < 0.001$; $F_{origin \times pop \times rhiz} = 16.2$, d.f. = 2,314, $P < 0.001$.

pre-cultured by conspecifics were significantly larger than in Montana soils pre-cultured by *F. idahoensis*. Sterilization of the soils eliminated these feedbacks. The negative effects of soil microbes are often enhanced in competitive environments[22], but we also found results almost identical to those in Fig. 2 for *C. maculosa* grown in competition with bunchgrasses in pre-cultured French or Montana soils (data not shown). Considered together, the results of the feedback experiments suggest that *C. maculosa* is able to modify the microbial community in invaded soils to its advantage. In contrast, *C. maculosa* is inhibited by a negative feedback in French soils, probably due to the accumulation of pathogens and potentially also due to adaptation of inhibitory microbial populations to antimicrobial compounds produced by *C. maculosa*[23]. However, the generality of these results should be interpreted with caution because of our choice to experiment with soils from sites with the maximal contrasting sterilization results.

In a field experiment designed to investigate plant–soil feedbacks in more realistic conditions in North America, we found that *C. maculosa* growing in locations that had been occupied by other *C. maculosa* plants for 3 years were over twice as large as other *C. maculosa* plants growing in sites occupied by the native grass, *Pseudoroegneria spicata* (0.67 ± 0.12 versus 0.30 ± 0.07 g, *t*-test, $P = 0.023$).

To examine descriptively the pre-culturing effects of different plant species on the composition of soil microbial communities from different regions, we conducted a comparative analysis of soil microbial community structure in the Central Massif (France) and Missoula (North America) soils using denaturing gradient gel electrophoresis of partial 16S and 18S rRNA gene fragments amplified by polymerase chain reaction (PCR) using the generally conserved primers 536fC and 907r. The results suggested that *C. maculosa* cultured European and North American microbial communities in different ways, and that the effects of *F. idahoensis* differed from that of *C. maculosa* (Supplementary Fig. 2). However, on the basis of these results alone we cannot determine whether *C. maculosa* stimulates or suppresses specific microbial populations.

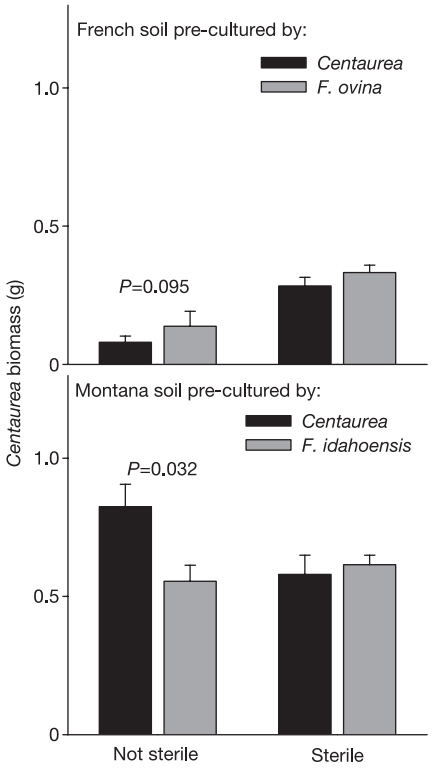

Figure 2 Total biomass of *C. maculosa* plants grown alone in European soil (Central Massif population) and North American soil (Missoula population) that had been pre-cultured by either *C. maculosa* or a *Festuca* species native to the place of soil origin. Plants were grown in soils either sterilized or not sterilized after pre-culturing. In a 3-way ANOVA (origin, species used for pre-culturing and sterilization as main effects) $F_{origin} = 159.7$, d.f. = 179, $P < 0.001$; $F_{culture \times species} = 0.30$, d.f. = 179, $P = 0.593$; $F_{sterilization} = 1.28$, d.f. = 179, $P = 0.260$; $F_{origin \times culture\ species} = 7.64$, d.f. = 179, $P = 0.007$; $F_{origin \times sterilization} = 13.99$, d.f. = 179, $P < 0.001$; $F_{culture\ species \times sterilization} = 6.03$, d.f. = 179, $P < 0.017$. *P*-values shown above paired bars indicate a significant difference in pre-culturing effects for those treatments.

Pathogen–plant relationships are often quite host-specific or vary substantially in their relative effects on different plant species[10,20,24]. If plants and pathogens co-evolve locally it would be expected that feedback between a plant species and soil microbes from its native range will be negative, and that exotic invaders may escape more pathogens than they acquire in their new habitat[5]. Mutualistic microbes can have host-specific associations and functions[25,26], but in contrast to the host-specific tendency of pathogenic microbes, many arbuscular mycorrhizal fungi tend to infect a broad range of hosts[25,27] (although there is evidence for specialization in the mutualistic benefits for the plant[28]), making it possible for invaders to use the native mycorrhizae of a new region. Therefore, the feedback of soil microbes from the invaded range of an exotic weed to the weed itself is likely to be neutral or positive because of the potential for the invader to accumulate mutualistic fungi in the absence of host-specific soil pathogens.

Our results support Klironomos' hypothesis[10] that some exotic invaders have escaped control by local soil pathogens, and may benefit from soil microbes in invaded regions. However, conclusive proof would require precise matching of seeds from exotic plant populations with their co-occurring soil microbes. We intentionally used seeds of *C. maculosa* and grasses from sources that did not match any specific population in an effort to eliminate any local bias that might confound continental comparisons. Until local exotic plant populations and their specific soil microbial communities are tested together, our understanding of escape from local pathogens remains incomplete.

Many mechanisms are involved in the expansion of exotic plant species. Our results provide comparative biogeographical experiments testing mechanisms for invasive success, and show that a switch from negative plant–soil microbial feedback in native habitats to positive plant–soil feedbacks in invaded habitats may contribute to the expansion of one of the world's worst invaders.□

Methods

Experiment 1

We collected soils from four sites in Europe and six sites in North America to assess biogeographical differences in the effects of soil microbes on *C. maculosa*, a native of Europe and an invasive weed in North America. The European sites were near Ales in the Central Massif, France (44° 51′ 0″ N; 0° 52′ 0″ E), near Briançon, France (44° 53′ 60″ N; 6° 39′ 0″ E), near Gap, France (44° 34′ 0″ N; 6° 4′ 60″ E), and at Pontamafrey, Italy (45° 43′ 60″ N; 7° 9′ 60″ E). The North American sites were Missoula, Montana (46° 52′ 23″ N; 113° 59′ 45″ W), the National Bison Range, Montana (47° 20′ 12″ N; 114° 13′ 46″ W), Montana State University Experimental Ranch, Montana (47° 03′ 30″ N; 113° 12′ 42″ W), Sapphire Mountains, Montana (46° 22′ 52″ N; 113° 56′ 56″ W), The Flathead Valley, Montana (47° 33′ 30″ N; 113° 42′ 60″ W) and Clearwater, Montana (47° 0′ 34″; 113° 23′ 53″ W). Soils were collected and immediately subjected to slow air-drying to mimic drying conditions that would occur during natural drought. After drying, one-half of the soils were treated by triple autoclaving on three successive days to kill soil microbes. Soil was mixed with sand (15:85 soil:sand mixture) within 4 weeks of collection into 525-cm^3 pots. *Centaurea* seeds from a single North American population for which soils were not used (Bozeman, Montana) were planted into all pots and grown for 140 days while being fertilized once every 2 weeks with 100 ml of 0.25 strength Hoaglands solution and watered every 2 days. After this period *Centaurea* plants were harvested, dried at 60 °C and weighed. Owing to an error in the autoclaving of grass rhizospheres in the Central Massif and Missoula, the sample sizes were reduced to 2–4 for each treatment site combination.

Experiments 2 and 3

Plant–soil microbe feedbacks were compared using soil from one *C. maculosa* population in Europe (Central Massif) and one in North America (Missoula). We chose to contrast these soils because they represented the extreme cases in each region. We

pre-cultured[1,2,10,20,21] the microbial communities from each site by growing either *C. maculosa* from the same region used in experiment 1, or a *Festuca* bunchgrass native to the region of soil origin, in pure soil. The two *Festuca* species (*idahoensis* and *ovina*) were chosen because they are similar in size and appearance and co-occur naturally with *C. maculosa* in their respective native lands. The use of a single *Festuca* species might have biased the relationship between the plant and microbes of a different continent in ways that might differ from a plant and microbes from the same region. Five plants were grown for 110 days in each of 40 4-litre pots: $n = 10$ for Montana soil with *C. maculosa*; Montana soil with *F. idahoensis*; French soil with *C. maculosa*; and French soil with *F. ovina*. After the 110-day pre-culturing period, half of the soil in each pot was triple-autoclaved on three successive days to kill the microbial community, and then the non-sterilized and sterilized soil from each 4-litre pot was used to inoculate two sterile 15:85 soil:sand mixtures and two non-sterile 15:85 soil:sand mixtures in 525 cm^3 pots. We used the soil in the 525 cm^3 pots for two experiments: one in which *C. maculosa* was grown alone in sterile and non-sterile soil, and one in which *C. maculosa* was grown with *F. idahoensis* as a competitor. In the no-competition experiment, *Centaurea* seeds were planted in all pots and grown for 91 days during which they were fertilized and watered as in experiment 1. In the competition experiment, *F. idahoensis*, which grows more slowly than *C. maculosa*, was planted first, and after 14 days *C. maculosa* seeds were planted in half of the pots and grown for 91 days. At the end of the experiments all plants were harvested, dried at 60 °C and weighed.

Experiment 4

We compared the post-removal effects of *C. maculosa* on the growth of conspecifics to those of the native grass *Pseudoroegneria spicata*. Ten *C. maculosa* and ten *P. spicata* were planted in random locations outdoors in the Deittart Experimental Gardens in April 2001. The site historically supported native grasslands. These plants were grown until 10 August 2003, when they were harvested aboveground. One *C. maculosa* was grown at each of the 20 sites from 10 August to 14 September 2003 when they were harvested aboveground, dried at 60 °C and weighed.

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Organization of genetic variation in individuals of arbuscular mycorrhizal fungi

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Arbuscular mycorrhizal (AM) fungi (Glomeromycota) are thought to be the oldest group of asexual multicellular organisms. They colonize the roots of most land plants, where they facilitate mineral uptake from the soil in exchange for plant-assimilated carbon[1]. Cells of AM fungi contain hundreds of nuclei. Unusual polymorphism of ribosomal DNA observed in individual spores of AM fungi inspired a hypothesis that heterokaryosis—that is, the coexistence of many dissimilar nuclei in cells—occurs throughout the AM fungal life history[2,3]. Here we report a genetic approach to test the hypothesis of heterokaryosis in AM fungi. Our study of the transmission of polymorphic genetic markers in natural isolates of *Glomus etunicatum*, coupled with direct amplification of rDNA from microdissected nuclei by polymerase chain reaction, supports the alternative hypothesis of homokaryosis, in which nuclei populating AM fungal individuals are genetically uniform. Intrasporal rDNA polymorphism contained in each nucleus signals a relaxation of concerted evolution[4], a recombination-driven process that is responsible for homogenizing rDNA repeats[5]. Polyploid organization of glomeromycotan genomes could accommodate intranuclear rDNA polymorphism and buffer these apparently asexual organisms against the effects of accumulating mutations.

Molecular phylogeny[6] and the fossil record[7] date Glomeromycota to the Ordovician period and indicate that AM-like fungi participated in the transition of early plants to the terrestrial habitat[8]. With no evidence of sexual reproduction, these fungi may represent an ancient asexual lineage that is much older than the asexual bdelloid rotifers[9]. The glomeromycotan reproductive mode, and their reputed position of ancient asexuals, could be verified by methods of phylogenetics and population genetics; however, this work cannot be accomplished until the genetic variation observed in individuals of AM fungi is explained.

Two models can explain the organization of genetic variation in AM fungi: first, the diverse rDNA variants may be distributed among different nuclei (heterokaryosis, Fig. 1a); or second, all