Root-associated fungal microbiota of nonmycorrhizal *Arabis alpina* and its contribution to plant phosphorus nutrition

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Most land plants live in association with arbuscular mycorrhizal (AM) fungi and rely on this symbiosis to scavenge phosphorus (P) from soil. The ability to establish this partnership has been lost in some plant lineages like the Brassicaceae, which raises the question of what alternative nutrition strategies such plants have to grow in P-impoverished soils. To understand the contribution of plant–microbiota interactions, we studied the root-associated fungal microbiome of *Arabis alpina* (Brassicaceae) with the hypothesis that some of its components can promote plant P acquisition. Using amplicon sequencing of the fungal internal transcribed spacer 2, we studied the root and rhizosphere fungal communities of *A. alpina* growing under natural and controlled conditions including low-P soils and identified a set of 15 fungal taxa consistently detected in its roots. This cohort included a Helotiales taxon exhibiting high abundance in roots of wild *A. alpina* growing in an extremely P-limited soil. Consequently, we isolated and subsequently reintroduced a specimen from this taxon into its native P-poor soil in which it improved plant growth and P uptake. The fungus exhibited mycorrhiza-like traits including colonization of the root endosphere and P transfer to the plant. Genome analysis revealed a link between its endophytic lifestyle and the expansion of its repertoire of carbohydrate-active enzymes. We report the discovery of a plant–fungus interaction facilitating the growth of a nonmycorrhizal plant under native P-limited conditions, thus uncovering a previously underestimated role of root fungal microbiota in P cycling.

Brassicaceae | microbiome | fungal endophyte | Helotiales | nutrient transfer

Comparable with the human microbiota, millions of microbes colonize plants and form complex communities on plant surfaces and in plant tissues. The interactions between the plant and its microbiota range from parasitism (detrimental to the host) to mutualism (mutually beneficial), and their outcome can be pivotal for plant performance. Plant-associated microbes can influence plant fitness by modulating plant growth, root architecture, nutrient acquisition, or drought and disease resistance (1–3). Thus, the plant microbiota can be seen as an extension of the plant genome in the sense that it can increase the plant's adaptation capacity (4). This is illustrated by the arbuscular mycorrhizal (AM) symbiosis established between land plants and Glomeromycota fungi, which is thought to have facilitated the adaptation of plants to a terrestrial life (5). It is estimated that 80% of the vascular plant species (6) receive phosphorus (P) and other nutritional elements from these fungi in exchange for photosynthates (7). The ability to form an AM symbiosis has been lost independently in several flowering plant lineages including the Brassicaceae family through the loss of essential symbiosis genes during evolution (8). Given the beneficial effect of AM fungi on plant P uptake, the question of whether nonmycorrhizal species thrive due to the exploitation of alternative P-mining strategies forms the basis of current research (9). In the context of the plant holobiont, i.e., the plant and all its microbial partners, models of plant nutrition need to account for these cross-kingdom interactions to be complete. Here, we integrate these concepts and study the role of root-associated fungi other than AM in plant P nutrition.

In some plant lineages, AM co-occurs with other mycorrhizal symbioses like ectomycorrhiza (woody plants), orchid mycorrhiza (orchids), and ericoid mycorrhiza (Ericaceae) (10). These associations can also promote plant nutrition; however, they have not been described in Brassicaceae. Endophytic microbes can promote plant P acquisition by different processes including P solubilization and mineralization (11) or transfer of P in the form of soluble orthophosphate. P transfer to their hosts was considered a hallmark of mycorrhizal fungi until recently. Two studies on binary root–fungus interactions showed that two endophytes—the Ascomycete *Colletotrichum tofieldiae* (12) and the Basidiomycete *Serendipita indica* (syn. *Piriformospora indica*) (13)—are able to transfer P to their nonmycorrhizal host *Arabidopsis thaliana*, promoting its growth under low-P conditions. *S. indica* was also demonstrated to participate in P uptake of maize plants depending on the expression of a fungal high-affinity phosphate transporter (14). These studies provided proof of concept for P transfer from fungi to nonmycorrhizal hosts; however, the ecological relevance of these interactions remains unclear as it is not known whether these endophytes can promote plant P uptake under native low-P soil conditions, and only *C. tofieldiae* was shown to be a natural inhabitant of *A. thaliana* roots. Descriptive and functional studies

Significance

Most terrestrial plants live in symbiosis with arbuscular mycorrhizal (AM) fungi and rely on this association to scavenge the macronutrient phosphorus (P) from soil. *Arabis alpina* thrives in P-limited alpine habitats, although, like all Brassicaceae species, it lacks the ability to establish an AM symbiosis. By studying the fungal microbiota associated with *A. alpina* roots we uncovered its association with a beneficial Helotiales fungus capable of promoting plant growth and P uptake, thereby facilitating plant adaptation to low-P environments.

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on the fungal microbiota of nonmycorrhizal plants are needed to improve our understanding of the ecological relevance of these interactions for plant nutrition at the holobiont level.

Although fungi represent a prominent part of the root microbiota where they can play important roles as pathogenic or beneficial partners, studies of Brassicaceae species have focused mainly on bacterial communities (1, 15–17). These studies have increased our knowledge of how root bacterial communities are shaped by environmental, edaphic, and host-related factors. Expansion of this knowledge to fungal communities is crucial as studies on the mycorrhizal host species poplar (18), sugar cane (19), and *Agave* (20) suggest that fungal and bacterial root communities respond differently to environmental cues. Microbiome studies focusing on taxonomical description have shown that fungi detected in plant tissues are often phylogenetically related to described plant pathogens or saprotrophs (3, 18). Comparative genomics analyses have shown that plant beneficial endophytic lifestyles can emerge from plant pathogenic or saprophytic fungal lineages through genome modifications often involving the expansion or contraction of gene families encoding carbohydrate active enzymes (CAZymes) involved in plant cell-wall degradation (21–23). Prediction of the ecological role of fungal root endophytes is thus challenging and requires more systematic studies associating endophyte isolation, in planta testing, and genomic investigation.

Arabis alpina (Brassicaceae) is a nonmycorrhizal perennial arctic-alpine herb growing in harsh and rocky environments (24) including P-impoverished soils (this study). In recent years it has emerged as a model for ecological and developmental studies, and its genome has recently been sequenced (25). The aim of the present study was to explore the root fungal microbiome of *A. alpina* and its contribution to plant P acquisition, following the hypothesis that root-associated fungi other than AM fungi can promote plant P uptake under natural and controlled low-P conditions. We used Illumina-based amplicon sequencing of the fungal taxonomical marker internal transcribed spacer 2 (ITS2) to describe the fungal microbiome in *A. alpina* roots (endosphere) and the rhizosphere (soil zone immediately surrounding the root) under greenhouse, common garden, and natural conditions. Microbiome variability analysis showed that root fungal communities were more robust in response to changing environments relative to the rhizosphere assemblages, leading to the description of a set of 15 fungal taxa consistently detected in *A. alpina* roots. Within this cohort we identified a fungal taxon belonging to the Helotiales order, exhibiting high abundance in the roots of wild *A. alpina* plants growing in an extremely P-limited soil. Successful isolation of a specimen from this taxon from *A. alpina* roots, followed by its reintroduction into the native P-limited soil after sterilization, promoted *A. alpina* growth and shoot P accumulation. In vitro studies further demonstrated that the fungus' contribution to plant growth involves transfer of inorganic phosphate to its host. Finally, fungal genome sequencing revealed an expansion of its repertoire of carbohydrate-active enzymes, which may be associated with its endophytic lifestyle. Cumulatively, these results provide evidence for a beneficial role of a hitherto unknown member of the root microbiota in *A. alpina* growth performance in low-P environments.

Results

Root-Associated Fungal Communities in *A. alpina* Were Unaffected by Host Genetic Variation. Our current understanding of the structure of the root microbiome in nonmycorrhizal Brassicaceae species rests primarily on bacterial communities, and information on the factors shaping root-associated fungal consortia is scarce. We studied fungal communities associated with *A. alpina* roots by sequencing the fungal ITS2 with primers ITS9/ITS4 as they showed a better recovery of low-abundance fungal diversity in comparison with other primers in a pilot experiment (*SI Appendix*, Fig. S1).

We assessed the effect of plant intraspecific variation on the structure of the root-associated fungal community by comparing four European *A. alpina* accessions (Fig. 1*A* and *SI Appendix*, Table S1) grown side by side at the Lautaret common garden (GAR-Lau). At harvest time, the accessions differed in size and developmental stage (*SI Appendix*, Fig. S2*D*) but shared similar root and rhizosphere fungal communities with comparable diversity (ANOVA $P > 0.05$) (*SI Appendix*, Fig. S2*B*) and structure, as observed in the principal coordinates analysis (PCoA) (*SI Appendix*, Fig. S2*A*) and verified by permutational multivariate analysis of variance (PERMANOVA) results ($P > 0.05$). This indicated that, under the assessed seminatural common garden conditions, host genetic variation fails to impact the root-associated fungal community in *A. alpina*. This contrasted with studies in which a small but significant contribution of the host genotype to structuring of bacterial root microbiomes was shown (1, 15, 26, 27) in host species including *A. alpina* (16). Our work suggests that, unlike bacterial communities, root-associated fungal communities are less or not at all affected by host genotypic differences in *A. alpina*. However, we cannot exclude that high within-genotype variability might be shading a small effect.

Soil and Environmental Cues Shape *A. alpina* Root and Rhizosphere Fungal Communities. We next assessed how soil and environment-associated factors shape fungal communities inhabiting bulk soil, the *A. alpina* rhizosphere, and the root endosphere (Fig. 1*A*). Under controlled greenhouse conditions (GrH) we studied the effect of three soils (*SI Appendix*, Table S2) with different geographical origins [Reckenholz (Rec) vs. Lautaret (Lau)] and P-fertilization regimes [Reckenholz soils with amended nitrogen (N) and potassium (K) (RecNK) vs. N, P, and K (RecNPK)]. on the fungal communities associated with *A. alpina* accession Pajares (PM).

Results showed that fungal alpha diversity (related to the number of taxa per sample) estimated by the Shannon diversity index was highly determined by the compartment type ($P = 2.10^{-10}$, 70% of variance, *SI Appendix*, Table S3), with lower values in root relative to rhizosphere and bulk soil compartments (ANOVA and Tukey's HSD, $P < 0.05$) (Fig. 1*C*), indicating the selection of a reduced number of fungal taxa entering the plant roots. Similarly, comparing the structure of those fungal communities (taxa present and their relative abundances) by permutational multivariate analysis of variance (PERMANOVA on Bray–Curtis dissimilarities) revealed that the major source of variation was the compartment type ($P = 10^{-4}$, 29% of variance, *SI Appendix*, Table S3).

Although neither the soil's geographical origin (Reckenholz vs. Lautaret) nor its P-fertilization regime (Reckenholz soils with NK vs. NPK amendment) significantly impacted the overall fungal diversity (ANOVA, $P > 0.05$), they did affect the structure of fungal communities. The effect of the soil's geographical origin (PERMANOVA, $P = 10^{-4}$, 21% of variance) decreased from the bulk soil (67% of variance) over the rhizosphere (49%) to the root compartment (30%), whereas the P-fertilization effect overall was smaller (PERMANOVA $P = 10^{-4}$, 6.3% of variance) and stable across the three compartments (14, 13, and 15% of variance in bulk soil, rhizosphere, and root communities, respectively) (*SI Appendix*, Table S3). This was evident in the PCoA on Bray–Curtis dissimilarities where greenhouse samples from the Lautaret soil (GrH-Lau) were grouped separately from samples obtained from the two Reckenholz soils (GrH-RecNK and GrH-RecNPK), which clustered more closely (Fig. 1*B*). This indicated that, under greenhouse conditions, the compartment type, the soil's geographical origin, and to a lesser extent its fertilization regime, all participated in the shaping of root-associated fungal communities. Collectively, these results suggested that fungal communities that were accommodated in the root endosphere were less diverse and less affected by soil changes than extraradical consortia.

We then assessed whether these fungal communities were affected by the plant growing environment and compared fungal communities established under controlled greenhouse conditions (GrH-Lau) with those established in the common garden under

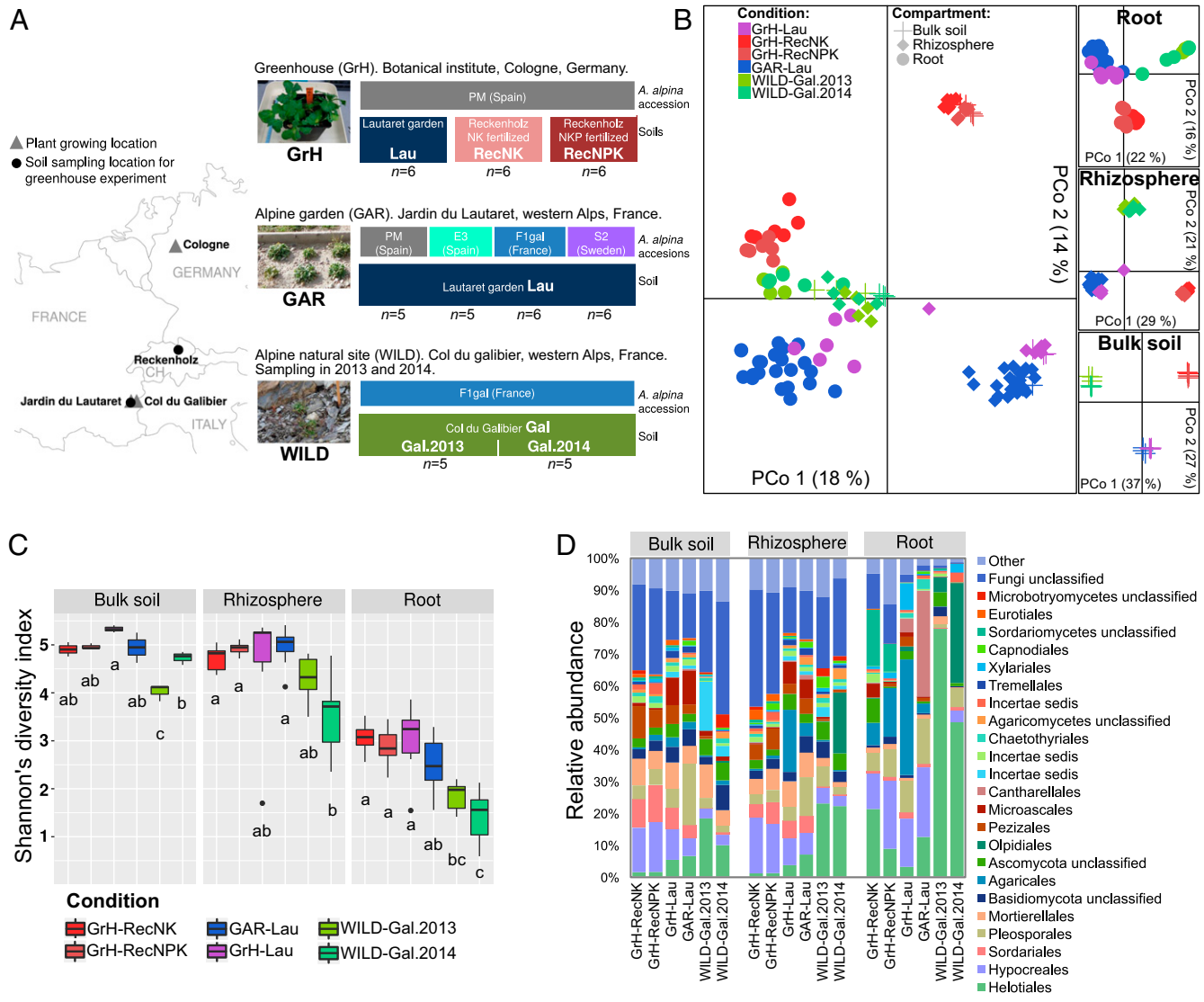

Fig. 1. Comparison of the fungal communities colonizing *A. alpina* roots and rhizosphere under greenhouse (GrH), common garden (GAR), and natural (WILD) conditions in different soils (RecNK, RecNPK, Lau, and Gal). (*A*) Experimental setup showing the different plant growing conditions. The geographic origin of the different *A. alpina* accessions is indicated in parentheses. More information about the soils and the accessions is given in *SI Appendix*, Tables S1 and S2, respectively. The number of biological replicates per condition (*n*) is indicated. (*B*) Principal coordinates analysis on fungal community differences (Bray–Curtis dissimilarities) in the different compartments and conditions. (*C*) Fungal alpha diversity estimated by Shannon's diversity index. Letters a–c indicate significant differences between conditions within each compartment (ANOVA and Tukey's HSD, $P < 0.05$). (*D*) Mean relative abundance of the major fungal orders in the different conditions and compartments: bulk soil, rhizosphere, and root. As the four *A. alpina* accessions studied exhibited similar fungal communities in the garden experiment (*SI Appendix*, Fig. S2), combined results for the four accessions are shown under the "GAR-Lau" condition.

alpine summer conditions (GAR-Lau) in the same soil (Lau) using *A. alpina* PM as host plant. Fungal communities established in the same soil but under the two contrasting environments, i.e., differing in altitude and climate, exhibited similar diversity (ANOVA $P > 0.05$, Fig. 1*C*) but differed in structure (PERMANOVA $P = 0.002$, 10% of variance). The effect of the environment type on fungal community structure increased from bulk soil (PERMANOVA $P = 0.1$) over rhizosphere soil (PERMANOVA $P = 0.002$, 21% of variance) to the root compartment (PERMANOVA $P = 0.003$, 30% of variance) (*SI Appendix*, Table S3). This strongly suggested that root-associated communities were affected to a greater extent by environmental changes than bulk soil communities, which remained largely unaffected.

Comparatively, common garden and wild *A. alpina* plants growing under similar alpine summer conditions but in different soils (GAR-Lau vs. WILD-Gal) also showed differences with lower diversity (Fig. 1*C*) and different community structure (PERMANOVA $P = 10^{-4}$, 20% of variance) in root and rhizosphere fungal communities from wild *A. alpina* (WILD-Gal) (*SI Appendix*, Table S3). This was observable in the PCoA with greenhouse and common garden samples from the Lautaret soil (GrH-Lau and GAR-Lau) clustering close and separating from samples from wild growing *A. alpina* (WILD-Gal) (Fig. 1*B*). Collectively, these results showed that under alpine conditions root-associated fungal communities still diverged according to the soil type.

Stability of Root and Rhizosphere Fungal Communities Across Varying Growth Conditions. We next performed a general analysis including all of the experiments to assess how fungal communities were affected by the plant growing condition. Six plant growing conditions were considered based on the environment and the soil in which the plants grew and included the confounding

effects of two different environments (greenhouse conditions: GrH and alpine summer conditions: GAR and WILD) and four soils (Lau, RecNK, RecNPK, and Gal). Since *A. alpina* accessions harbored similar fungal communities (*SI Appendix*, Fig. S2), samples from the different accessions were grouped under the same plant growing condition, GAR-Lau.

In all plant growing conditions, *A. alpina* root-associated (i.e., root and rhizosphere) fungal communities were dominated by ascomycetes (58% of the fungal reads) belonging mostly to the orders Helotiales, Hypocreales, Pleosporales, and Sordariales. Basidiomycetes (18%), unclassified fungi (14%), zygomycetes (4.4%), and chytridiomycetes (4.1%) were less abundant. As expected for a nonmycorrhizal plant, glomeromycetes that include the AM fungi were rarely detected (0.04%). While the Helotiales (24% of the fungal reads in roots) and Cantharellales (16%) orders were enriched in root samples, Mortierellales (6% of the fungal reads in the rhizosphere), Sordariales (4.2%), and an unclassified basidiomycete taxon (4.1%) were enriched in the rhizosphere (paired *t* test $P < 0.05$) (Fig. 1*D*).

Comparison of fungal communities at the operational taxonomic unit (OTU) level showed again that the compartment type was the main driver of fungal alpha diversity ($P = 2.10^{-16}$, 72% of variance) with a bigger effect than the plant growing condition ($P = 10^{-16}$, 9.4%) (Fig. 1*C*) (*SI Appendix*, Table S3). Comparison of fungal community structure by PCoA showed a separation between root and soil (rhizosphere and bulk soil) samples mainly along the first axis (18% of variance) and a separation between plant growing conditions mainly along the second axis (14% of variance) (Fig. 1*B*). Within the three compartments (root, rhizosphere, and bulk soil) fungal communities clustered according to the geographic origin of the soil: samples from soil Gal (WILD-Gal) separated from soil Lau (independently of the environment) and from Reckenholz soils RecNK and RecNPK, which clustered together (Fig. 1*B*). PERMANOVA analysis indicated that the major source of variation in community structure was the plant growing condition ($P = 10^{-5}$, 32% of variance) and not the compartment type ($P = 10^{-5}$, 16%) (*SI Appendix*, Table S3). This contrasted with the PCoA (Fig. 1*B*), which hinted to a stronger effect of the compartment type. This can be explained by the fact that the PCoA captured only a part of the communities' differences. Interestingly, there was a significant interaction between the compartment type and the plant growing condition (PERMANOVA, $P = 10^{-5}$, 16%, *SI Appendix*, Table S3), suggesting that root, rhizosphere, and bulk soil fungal communities responded differently to varying plant growing conditions. Indeed, PERMANOVA analysis within each compartment showed that the effect of the plant growing condition on the fungal community steadily decreased from the bulk soil ($P = 10^{-5}$, 83%) over the rhizosphere ($P = 10^{-5}$, 59%) to the root ($P = 10^{-5}$, 49%) (*SI Appendix*, Table S3), suggesting that root fungal communities were more robust relative to extraradical assemblages. In sum, this analysis at a wide scale, including contrasting environments and soils, showed that, although the microhabitat type (bulk soil, rhizosphere, or root compartment) is the main driver of fungal alpha diversity, the plant growing condition is the main factor structuring root-associated fungal communities, i.e., determining the taxa present and their abundances. Moreover, it suggested that, compared with rhizosphere communities, fungal communities living within *A. alpina* roots were less affected by changes in the plant growing condition.

Fungal Taxa Consistently Found in *A. alpina* Roots. Following the postulate that commonly occurring organisms play critical roles in their habitat, we aimed at identifying fungal taxa that consistently colonized *A. alpina* roots, hypothesizing that they promote plant growth and/or P uptake. We identified 15 highly conserved fungal OTUs with a high prevalence in roots (i.e., present in at least 85% of the root samples) (Fig. 2*A*). It comprised one zygomycete (*Mortierella elongata*, OTU00045), one basidiomycete (*Ceratobasidiaceae* sp., OTU00008), and 13 ascomycetes belonging to the Helotiales (4 OTUs), the Pleosporales (4 OTUs), the Hypocreales (3 OTUs), the Sordariales (1 OTU), and one unclassified order (OTU00015). On average, this cohort represented 43% of the fungal reads detected in *A. alpina* roots with values ranging from 10 to 93% (Fig. 2*B*). Of these 15 highly conserved root OTUs, 7 were enriched in plant roots in comparison with the rhizosphere (paired *t* test, $P < 0.05$). They included two unclassified species belonging to the Pleosporales (OTU00007, 92% prevalence, 1.1% relative abundance) and the Ceratobasidiaceae (OTU00008, 90%,

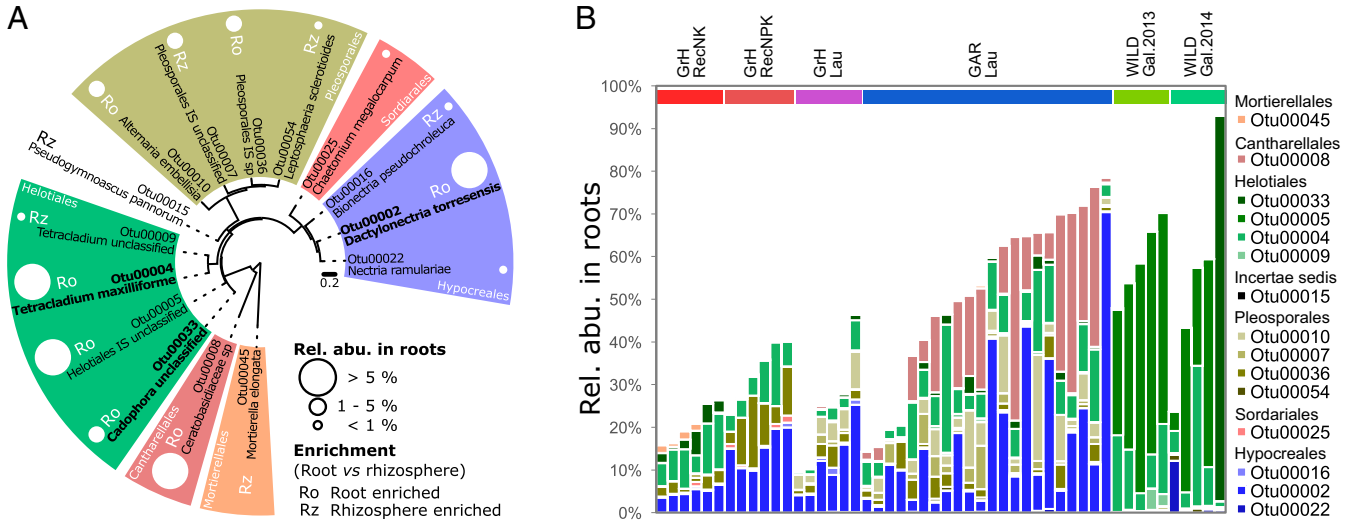

Fig. 2. Fungal taxa consistently found in *A. alpina* roots (>85% prevalence across all root samples). (*A*) Maximum-likelihood phylogenetic tree of the highly conserved root OTUs. The representative ITS2 sequences from the OTUs were aligned using Muscle (28) and used for tree inference in PhyML (29) with a GTR+I+γ model with optimized parameters. Fungal orders are depicted with different colors; white circles indicate the average relative abundance (Rel. abu.) of the OTU in root samples. Root (Ro)- or rhizosphere (Rz)-enriched OTUs are indicated (comparison root vs. rhizosphere relative abundance, paired *t* test, $P < 0.05$). OTUs with 100% prevalence are shown in boldface type. (*B*) Relative abundance of the 15 highly conserved root OTUs in each root sample. The data are given in Dataset S4.

6.5%); *Alternaria embellisia* (OTU00010, 88%, 2.8%); *Dactylonectria torresensis* (OTU00002, 100%, 11%); and three Helotiales including *Tetracladium maxilliforme* (OTU00004, 100%, 8.5%), a *Cadophora* OTU (OTU00033, 98%, 3%), and an unclassified OTU (OTU00005, 86%, 7%) (Fig. 2*A*). None of these highly conserved root OTUs was related to (*i*) AM, ectomycorrhizal, or orchid mycorrhizal fungi known to facilitate plant nutrient uptake or to (*ii*) fungal endophytes *S. indica* (syn. *P. indica*) or *C. tofieldiae*, which were described to transfer P to nonmycorrhizal plants. Four of these OTUs belonged to the Helotiales order known to encompass ericoid mycorrhizal fungi such as *Oidiodendron maius* but also plant pathogens such as *Rhynchosporium secalis*. Three of these four Helotiales OTUs showed enrichment in roots and high abundance especially under natural conditions (Fig. 2*B*).

Helotiales Fungus F229 (OTU00005) Promotes *A. alpina* Growth and Shoot P Accumulation. Helotiales OTU00005 exhibited a high relative abundance in the roots of wild *A. alpina* plants from Col du Galibier (45% in WILD-Gal.2013 and 23% in WILD-Gal.2014 samples, *SI Appendix*, Fig. S5*B*), where the host plants grew on an extremely low-P soil (soil Gal, 3.7 mg/kg plant-available P) while maintaining high shoot P concentration (*SI Appendix*, Fig. S5*A*). We subsequently identified in our Cologne Culture Collection of Root-Associated Fungi (CORFU) seven isolates that were recovered from *A. alpina* collected at Col du Galibier and belonged to this OTU (Dataset S1). The full-length ITS sequences of the isolates shared 99–100% similarity, and their ITS2 regions showed 99–100% similarity to the representative ITS2 sequence of OTU00005. Blast analysis revealed that the ITS sequences of these isolates were highly similar to other Helotiales root endophytes isolated from the Brassicaceae species *Microthlaspi perfoliatum* growing in the south of Spain (30), thus reflecting a recurrent presence of these Helotiales fungi in roots of Brassicaceae (*SI Appendix*, Fig. S3).

To address the significance of fungal root colonization for plant P uptake, the fungus with CORFU identifier F229 (hereafter named F229), belonging to OTU00005, was selected for further in planta experiments in gnotobiotic Murashige and Skoog (MS) agar systems. F229 promoted growth of *A. alpina* F1gal and PM roots under low-P conditions and left plants unaffected in high-P conditions (*SI Appendix*, Fig. S4). In contrast, another six fungal isolates not belonging to OTU00005 (CORFU F226, F248, F247, F91, F83, F222), screened in different experiments, all exerted a negative effect on plant root and/or shoot growth in at least one of the P conditions (*SI Appendix*, Fig. S4). Fourteen days post-inoculation (dpi) on its natural host *A. alpina* F1gal, F229 asymptomatically colonized the plant roots (*SI Appendix*, Fig. S6 *B* and *C*) with equal colonization at low-P (100 μM P MS agar, 89.6% of colonized roots) and high-P (1,000 μM, 88.6% of colonized roots) conditions (χ^2 test $P = 0.87$). However, under low-P conditions, fungal inoculation significantly increased root length (+12%, *t test* $P = 0.02$) and root surface area (+19%, *t* test $P = 0.001$) while leaving shoot biomass (*t* test $P > 0.05$) and shoot P concentration (*t* test $P > 0.05$) unchanged (*SI Appendix*, Fig. S6 *A* and *B*). A neutral effect on root growth was apparent under high-P conditions (1,000 μM P, 14 dpi) (*SI Appendix*, Fig. S6*A*). Similarly, a second isolate (CORFU F240), also assigned to OTU00005 and exhibiting 100% ITS sequence similarity with F229, also promoted root growth of *A. alpina* F1gal under low-P conditions (*SI Appendix*, Fig. S6*D*).

We next assessed the effect of F229 on the growth of its natural host *A. alpina* F1gal under native P-limited soil conditions (Fig. 3*A*). At 28 dpi in gnotobiotic microcosms filled with autoclaved soil from the Col du Galibier (soil Gal), F229 fully colonized plant roots (100% of colonized roots) inter- and intracellularly (Fig. 3*B*, *a–d*). Vital staining of plant and fungal membranes indicated viability of host and fungal cells during intracellular accommodation (Fig. 3*B*, *d*), indicating a biotrophic interaction between the partners. While addition of heat-killed fungal suspension negatively affected plant growth, fungal inoculation translated into 52% higher shoot biomass (Mann–Whitney test $P = 3.10^{-13}$) and

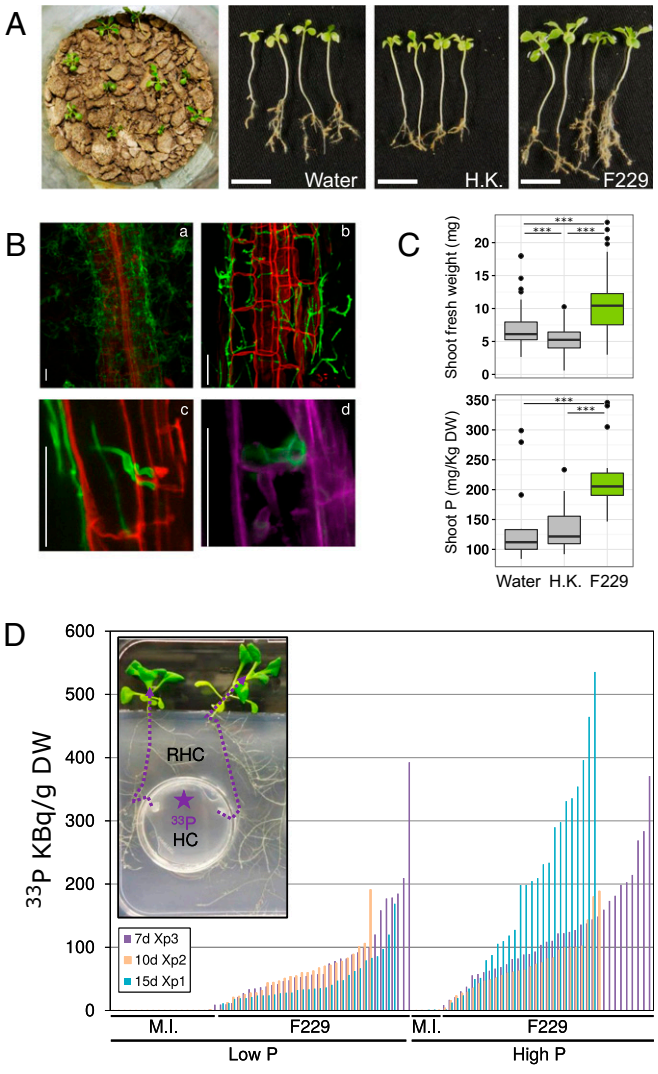

Fig. 3. Fungus F229 (OTU00005) increases *A. alpina* growth and P content under native low-P soil conditions and is capable of hyphal P transfer to the root in vitro. (*A*) *A. alpina* F1gal growth in sterile soil microcosm upon water addition (Water), addition of heat-killed fungus (H.K.), and inoculation with F229 (F229) ($1.32 \pm 0.8 \times 10^4$ propagules per microcosm) at 28 dpi. (Scale bars, 1 cm.) (*B*) Inter- and intracellular fungal root colonization in sterile soil microcosms visualized by confocal microscopy after staining the fungal cell wall with WGA-Alexa (green, *a–d*), the plant cell wall with propidium iodide (red, *a–c*), and the cellular membranes with FM4-64 (purple, *d*). (Scale bars, 30 μm.) (*C*) Effect of F229 inoculation on shoot fresh weight and shoot P concentration in sterile soil microcosms. The experiment was repeated four times including the Water and F229 treatments and three times including also the H.K. treatment, with three to four microcosms per treatment; similar results were obtained, and compiled results from the four experiments are shown here. Shoot weight was measured on individual plants ($n \geq 56$) whereas all of the shoots from one microcosm were pooled to measure shoot P content by ICP-MS ($n \geq 9$). Asterisks indicate significant differences between the treatments based on the Mann–Whitney test ($P < 0.05$). (*D*) In vitro transfer of ^{33}P orthophosphate to the plant by F229. The F229 and *A. alpina* F1gal plants were grown on low-P (100 μM P) or high-P (1,000 μM P) MS medium in a two-compartment system. ^{33}P was added to the fungal HC, and after 7 (experiment 3), 10 (experiment 2), or 15 (experiment 1) days, ^{33}P incorporation into the plant shoot growing in the RHC was measured by scintillation counting of individual plants. No fungus was added to the fungal compartment in the mock inoculated treatments. Bars represent individual samples.

61% higher shoot P concentration (Mann–Whitney test $P = 2.10^{-4}$) compared with the water control (Fig. 3*C*). The shoot P levels were still lower than observed in their wild-growing counterparts (WILD-Gal treatments, *SI Appendix*, Fig. S5). We could imagine two possible reasons explaining this discrepancy: (*i*) The young plants in the microcosms accumulated less P in their vacuoles, the primary intracellular compartments for inorganic phosphate, than the much older wild plants or (*ii*) differences in properties of soil in microcosms relative to soil at Col du Galibier limited P uptake, which was partially alleviated through root colonization with F229. In sum, these results corroborate the hypothesis of a beneficial effect of this endophyte on host growth and P acquisition under native low-P soil conditions.

F229 Translocates P to Its Host in Vitro. Fungi can promote plant P acquisition by different mechanisms like P solubilization, P mineralization, or hyphal P transfer. We wanted to know whether F229 is capable of hyphal transport of radiolabeled ^{33}P to its host. Using a two-compartment agarose system limiting radiotracer diffusion, we observed that ^{33}P added to the hyphal compartment (HC) could be traced to the plant shoot in the root and hyphal compartment (RHC), with both compartments connected only by fungal hyphae crossing the physical barrier (Fig. 3*D*). Fungal colonization was restricted to the root as the fungus was never detected in the plant shoot (stem or leaves, *SI Appendix*, Fig. S7). ^{33}P translocation across the diffusion barrier was blocked by Benomyl (*SI Appendix*, Fig. S8), a compound that inhibits microtubule formation and intracellular transport in fungi (31), which indicated that hyphal P transport was an active fungal process rather than mediated by diffusion. Moreover, hyphal ^{33}P translocation to the plant shoot was detectable as early as 7 d after ^{33}P addition and was independent from low- or high-P conditions. This suggests that in F229 ^{33}P transfer to the host is not regulated by P availability, which stands in contrast to what was shown for *C. tofieldiae* (12). These data suggest that plant growth promotion by F229 under low-P soil conditions involves hyphal transfer of P into its host.

The F229 Genome Encodes Two High-Affinity Phosphate Transporters. Cellular uptake of nutrients, maintenance of cellular nutrient homeostasis, and ion transfer across cellular (endo)membranes in fungus–plant symbioses involves high- and low-affinity ion transporters. To obtain insight into the molecular mechanisms underlying transport of inorganic phosphate in F229, we performed genome-wide analysis to identify fungal phosphate transporters. PacBio sequencing of the F229 genome produced a final assembly of 39 contigs with an estimated genome size of ∼85 Mb (Dataset S2). The final genome version showed a high level of completeness with a high coverage of core fungal (98.7%, FUNYBASE gene set) and eukaryotic genes (99.2%, Cluster of Essential Genes gene set). We aimed to identify homologs of six proteins that play a role in phosphate transport in the yeast *Saccharomyces cerevisiae*. One is the major proton-coupled high-affinity phosphate transporter Pho84, three transport phosphate with low affinity into cells (Pho87, Pho90, Pho91), and the fifth and sixth proteins (Pho 88 and Pho89) are utilized under specialized conditions (32). Two genes in the F229 genome (g8711.t1 and g16086.t1) encode proteins sharing high similarity with *S. cerevisiae* Pho84 and with fungal high-affinity phosphate transporters involved in P translocation from fungus to plant. One gene (g3490.t1) encodes a protein sharing similarity with Pho87, Pho90, and Pho91, and a last gene (g6261.t1) encodes a protein sharing similarity with Pho88. The gene encoding the Na^+-dependent high-affinity Pho89 could not be identified (*SI Appendix*, Fig. S9). In sum, these results show that the F229 genome encodes a set of phosphate transporters potentially enabling phosphate uptake and translocation to its host plant.

The Endophytic Lifestyle of F229 is Associated with the Expansion of its CAZyme Repertoire. We aimed at identifying genomic characteristics associated with the F229 endophytic biotrophic lifestyle. A five-gene phylogenetic analysis on F229 and 50 other ascomycetes with available genome information confirmed the placement of the fungus within the Leotiomycetes class and the Helotiales order (*SI Appendix*, Fig. S10). The classification of the fungus at the family or genus level was not possible as the taxonomy within the Helotiales order is still unclear. The closest relatives (with sequenced genome) of F229 are plant pathogens *Marssonina brunnea* f. sp. *multigermtubi* and *Rhynchosporium* species *R. secalis*, *R. commune*, and *R. agropyri*, suggesting that the endophytic lifestyle of F229 could have evolved from an ancestral plant pathogenic lifestyle (Fig. 4*A*). Similarly, the ITS-based phylogenetic analysis including more fungal isolates suggests that F229 belongs to a lineage of root endophytic fungi that diverged from related pathogenic *Rhynchosporium* and *Pyrenopeziza* species (*SI Appendix*, Fig. S3), but more Helotiales genomes are needed to properly assess this hypothesis using more robust phylogenetic analyses.

The Helotiales order encompasses fungi with contrasting lifestyles including plant beneficial fungi, plant pathogens, and saprotrophs (Fig. 4*A*). We used a comparative genomics approach on CAZyme repertoires to identify genomic signatures associated with a biotrophic lifestyle and plant-beneficial effects within this order. Comparison of CAZyme class profiles of 11 Helotiales fungi revealed large similarities between plant beneficial fungi that clustered together (Fig. 4*B*). The cluster including F229, the poplar endophyte *Phialocephala scopiformis*, and the ericoid mycorrhiza *O. maius* was characterized by a higher number (*t* test $P < 0.01$) of modules of glycoside hydrolases (GH) (average number of 427 in plant-beneficial fungi, 349 in F229, and 256 in the other fungi), carbohydrate-binding modules (CBM) (103, 109, and 77), carbohydrate esterases (CE) (194, 160, and 105), glycosyltransferases (GT) (128, 122, and 102), and auxiliary activities (AA) (137, 135, and 83), indicating an overall larger CAZyme repertoire in the genomes of plant beneficial Helotiales in comparison with plant pathogenic and saprotrophic Helotiales (Dataset S3). Comparison of CAZyme family profiles showed similar results (*SI Appendix*, Fig. S11). Twenty-two CAZyme families were significantly more abundant in plant beneficial fungi in comparison with the other fungi (*t* test $P < 0.01$) (Fig. 4*C*). Notably, this concerned families associated with plant cell-wall degradation, acting on hemicellulose (GH31, GH29, CE1, CE7, CE10), or in the transformation of lignocellulosic compounds (AA7). Three GHs associated with fungal cell-wall degradation (GH20, GH72, GH76) were also more abundant in plant beneficial fungi. Comparison of CAZyme classes (*SI Appendix*, Fig. S12*A*) and selected CAZyme families (*SI Appendix*, Fig. S12*B*) across 51 ascomycetes genomes showed no clustering corresponding to fungal lifestyle differences, indicating that the observations made within the Helotiales are lineage-specific. Collectively, these data suggest that the endophytic behavior of F229 is associated with the enlargement of its CAZyme repertoire and particularly with protein families associated with plant cell-wall degradation.

Discussion

Low availability of phosphate is a major factor constraining plant growth, performance, and metabolism in many natural and agricultural soils worldwide due to the poor solubility and mobility of soil P. The AM fungi have been shown to benefit plant productivity due to their contribution to plant nutrition, especially in nutrient-poor soils (10). The predominant function of AM fungi is attributed to increased host plant phosphate uptake as a consequence of a phosphate transport mechanism (7). Brassicaceae species lack the ability to establish an AM symbiosis, and, to fully comprehend how these plants thrive in P-limited habitats, it is required that we improve our understanding of their

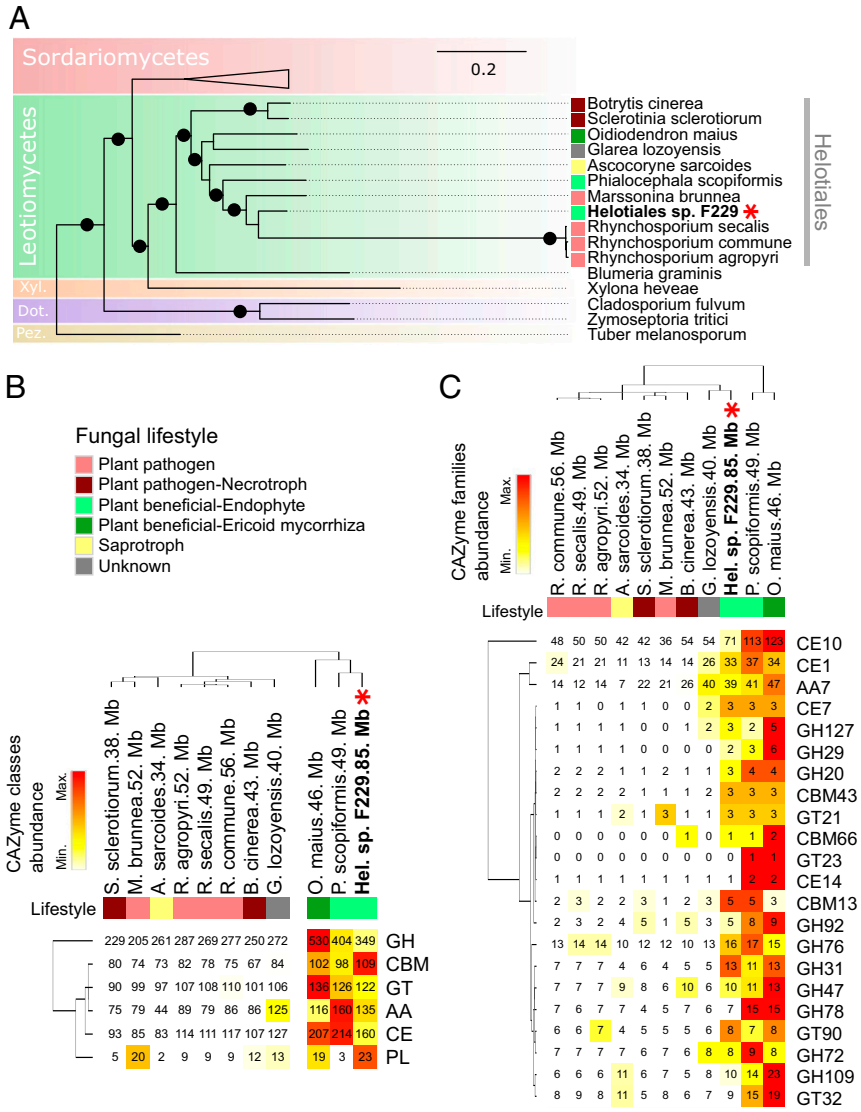

Fig. 4. The endophytic lifestyle of F229 is associated with the expansion of its CAZyme repertoire. (*A*) Maximum-likelihood phylogenetic tree inferred from five housekeeping genes (28S, 18S, Rpb1, Rpb2, EF1alpha). Bootstrap values >0.75 are indicated with a black dot. *Laccaria bicolor* sequences were used for tree rooting. Helotiales with plant beneficial, plant pathogenic, or saprophytic lifestyles are indicated; the key is given in *B*. The full tree is shown in *SI Appendix*, Fig. S10. (*B*) Comparative analysis of CAZyme repertoires in the genome of F229 and related Helotiales with plant beneficial, plant pathogenic, or saprophytic lifestyles. Hierarchical clustering on the abundance of CAZyme classes within the Helotiales. AA, auxiliary activities; CBM, carbohydrate-binding module; CE, carbohydrate esterase; GH, glycoside hydrolase; GT, glycosyltransferase; PL, polysaccharide lyase. (*C*) Hierarchical clustering on the abundance of selected CAZyme families within the Helotiales. Only families showing a significantly higher abundance in plant-beneficial fungi are shown (*t* tests, $P < 0.01$). In *B* and *C*, the color scale depicts standardized values for each module. Fungal genome sizes are indicated after their name. F229 is shown in boldface type with an asterisk.

fungal microbiota. Here we close this gap by investigating structural and functional properties of the fungal microbiota associated with the roots of the nonmycorrhizal Brassicaceae species *A. alpina*. We have chosen *A. alpina* because it naturally grows in low-P habitats, and, in contrast to short-lived annuals like the model species *A. thaliana*, its perennial pattern of growth and development gives longer time periods for microbial communities to establish in and around the roots.

Variability and Stability of the *A. alpina* Root Fungal Microbiome. Our study of the factors shaping root-associated fungal communities showed that fungal alpha diversity is determined mainly by the microhabitat type, i.e., bulk soil, rhizosphere, or root compartment, dropping dramatically in the root (Fig. 1*C*). This observation further sustains the view that colonization of the root endosphere is restricted to a reduced number of fungal taxa that have the ability to cross the selection filters imposed by the host. This hypothesis is supported by previous studies on root microbial communities that have shown a similar diversity pattern in bacterial assemblages in *A. alpina*, *A. thaliana*, and rice (1, 15, 16, 26) and in fungal communities of *Agave* (20). Furthermore, the microhabitat type also affected the structure of these fungal communities, i.e., the taxa present and their relative abundances (Fig. 1*B*). Overall, the fungal microbiome associated with roots (root and rhizosphere) of nonmycorrhizal *A. alpina* was dominated by ascomycetes, as was shown for mycorrhizal poplar and *Agave* (18, 20), and which is likely to be predetermined by the majoritarian presence of ascomycetes in soil (33). Still, root endosphere communities systematically differed from rhizosphere communities located millimeters apart (Fig. 1*B*); they were enriched in Helotiales and Cantharellales fungi and depleted in Mortierellales and Sordariales (Fig. 1*D*). Surprisingly, there was no clear similarity in

the pattern of enriched and depleted fungal orders in *A. alpina* and mycorrhizal *Agave*, poplar, and sugarcane roots (18–20), suggesting a low level of conservation of fungal microbiome patterns across distantly related plant host species.

Adding to the strong microhabitat effect, the soil geographical provenance was the second largest driver of fungal community structure under controlled greenhouse conditions. Soil fungal communities show strong biogeographical patterns shaped by local climatic and edaphic factors (33–35) and thus strongly diverge from the "everything is everywhere" postulate suggested for microbial communities (36). The soil's P-fertilization regime also impacted the structure of fungal communities, albeit to a lesser extent than the soil geographical origin (Fig. 1*B*). This indicated that long-term P fertilization of the RecNPK soil (37) shifted soil fungal communities. Differences in root communities could be the consequence of these changes, but we cannot exclude that they could be linked to changes in the plant nutritional status associated with fertilization. Such changes could lead to differences in root-associated communities by altering the root exudation profile and/or morphology, similarly to the AM symbiosis that is confined to conditions in which the plant is P-starved (7, 37). Even when growing in the same soil, fungal communities described under controlled greenhouse conditions differed from those established under alpine summer conditions (Fig. 1*B*). This environment effect increased from the bulk soil to the root compartment, suggesting that root-associated communities were more responsive to environmental change than bulk soil communities, which remained roughly alike. Low night temperatures (prevalent under alpine summer conditions) affect plant defense mechanisms as shown in *Arabidospsis* (38), which could directly impact endophytic fungal communities.

Our analysis including all of the plant growing conditions studied showed that at this wide scale it was no longer the microhabitat type, i.e., the compartment type, but the plant growing condition that was the main factor shaping fungal communities. This is consistent with what has been described in *Agave* (20) and poplar (18), where the plant biogeography was the major source of variation in fungal and bacterial communities. Interestingly, root communities were less affected by the plant growing condition than soil communities (rhizosphere and bulk soil) (Fig. 1*B*) as observed in *Agave* (20). This pointed to the existence of a set of fungal taxa consistently colonizing *A. alpina* roots in contrasting growth conditions. Our study revealed a highly conserved set of 15 OTUs that was dominated by ascomycetes (Fig. 2*A*) and represented up to 93% of the fungal reads in *A. alpina* roots (Fig. 2*B*). Seven OTUs of this core microbiome were significantly enriched in the root endosphere in comparison with the rhizosphere (Fig. 2*B*), suggesting not only that these taxa were able to cross the selection barrier imposed by the root, but also that they reached a higher abundance within the root endosphere, implying some degree of adaptation to this niche. Although most of the identified OTUs could not be classified at the species level, we could identify the *A. embellisia* and *D. torresensis* species. Both *Alternaria* and *Dactylonectria* genera are known to contain a high number of plant pathogenic species albeit with no evidence of pathogenicity in *A. alpina*. Interestingly, three closely related Helotiales OTUs were identified as highly conserved, root-enriched, and highly abundant in wild-growing plants (Fig. 2*A*). While Helotiales fungi represented 24% of the *A. alpina* root microbiome, they were not commonly found in root microbiomes of the mycorrhizal hosts *Agave* (20), poplar (18), or sugar cane (19). However, they were found to dominate the root microbiome of mycorrhizal Ericaceae species growing under similar cold and nutrient-limited conditions as *A. alpina* (39), suggesting that this could be a specificity of plants growing in such harsh environments. The Helotiales order is not well studied, and its phylogeny is still obscure. Although Helotiales fungi have often been isolated from plant roots, their ecological functions remain largely unknown (40). Our results indicating a plant growth-promoting effect of two Helotiales isolates (F229 and F240, OTU00005) in vitro (*SI Appendix*, Fig. S6) in combination with the high relative abundance of the corresponding OTU in *A. alpina* plants growing under P-poor natural conditions (*SI Appendix*, Fig. S5) suggests that this taxon could facilitate plant P uptake in its natural environment. Further studies are needed to investigate more *A. alpina* natural populations at different locations to define the biogeographic distribution of this beneficial plant–fungus association.

Endophytic Helotiales Fungus F229 Promotes Growth and P Acquisition in *A. alpina*. While most root-associated microbes compete with the plant and with each other for essential nutrients, some may have the potential to positively affect plant nutrition and growth. Helotiales fungal isolate F229 belonging to OTU00005 was isolated from *A. alpina* roots growing at the Col du Galibier natural site characterized by low-P availability in soil. The fungus exhibited biotrophic endophytic growth as it asymptomatically colonized plant roots inter- and intracellularly (Fig. 3*B*) rather than killing root cells during the infection process, coinciding with the root enrichment of the corresponding OTU (Fig. 2*A*). F229 was able to translocate P to the plant under high- and low-P conditions on MS agar (Fig. 3*D*). However, no increase in shoot P content was observed under those conditions (*SI Appendix*, Fig. S6). One plausible explanation is that as observed for AM symbiosis, translocation of P by the fungus does not necessarily translate into increased P content in the plant since the plant can tune-down the direct P uptake pathway and use the mycorrhizal pathway instead (41). When reintroduced into its native low-P soil fungus, F229 successfully colonized plant roots and enhanced shoot growth and shoot P concentration (Fig. 3*C*) through an active process (Fig. 3*C*). Our results stay in accordance with a role of F229 in extending the potential range of plant nutrient absorption in low-P soils and potentially also in P-rich habitats. In addition to increasing the absorptive surface area of the host plant root system, hyphal P translocation mediated through the activity of phosphate transporters encoded in the fungal genome would enable access to soil P sources otherwise unavailable to the plant (Fig. 3*D* and *SI Appendix*, Fig. S9). We cannot exclude, however, P delivery from fungus to host as a consequence of lysis of fungal cells, a mechanism that was proposed for nutrient transfer in orchid mycorrhizae (42). Overall, our work on F229 (P transfer) and studies on *S. indica* (P transfer) (13, 14), *C. tofieldiae* (P transfer) (12), *Heteroconium chaetospira* (N transfer), (43) and *Metarhizium robertsii* (N transfer) (44) provide accumulating evidence that fungus-to-plant nutrient transfer, generally assigned to classical mycorrhizal symbioses (10), is more common than previously thought.

Plant-colonizing fungi rely on hydrolytic enzymes including CAZymes for degradation of the plant cell wall and penetration into the host tissue (45, 46), and changes in the CAZyme repertoires have been associated with lifestyle changes in plant-associated fungi (21, 22). The evolution from pathogenic ancestors toward the beneficial endophytic lifestyle of F229 was accompanied by the enlargement of its CAZyme arsenal (Fig. 4). This contrasts with observations of ectomycorrhizal fungi where the transition from a saprophytic to an endophytic lifestyle was associated with a reduction of the number of genes encoding plant cell-wall–degrading enzymes (21, 22). This discrepancy has been noted in other root endophytes including mycorrhizal fungi (21, 45, 47). One explanation is that the arsenal of enzymes potentially involved in plant cell-wall degradation is a genomic indicator of saprophytic growth in plant debris in soil, making these fungi less dependent on their host for photosynthetically derived carbon.

In conclusion, by studying the fungal microbiota associated with *A. alpina* roots, we have uncovered a beneficial Helotiales fungus capable of promoting plant growth and P uptake and thereby potentially facilitating plant adaptation to low-P environments.

Materials and Methods

Plant Growth and Sample Collection. We analyzed the fungal communities colonizing the roots and rhizosphere of 60 *A. alpina* plants growing (*i*) under GrH in three different soils (Lau, RecNK, and RecNPK; soil characteristics are given in *SI Appendix*, Table S2); (*ii*) under alpine summer conditions in a common garden in the French Alps (GAR); and (*iii*) at a natural site in the French Alps (WILD) over 2 y (2013 and 2014) (Fig. 1*A*). Four *A. alpina* accessions (PM, E3, S2, and F1gal) originating from different European locations with different soil characteristics (*SI Appendix*, Table S1) were included in the common garden experiment to assess the contribution of the plant genotype to the structuring of the root-associated fungal community. Root and rhizosphere compartments from five to six replicates per condition were collected. The rhizosphere was sampled as the soil tightly adhering to roots, and root samples were enriched in endophytic fungi by detaching surface-adhering fungi through sonication (1). Three unplanted bulk-soil samples were included in each experiment. The detailed procedure is given in *SI Appendix, Materials and Methods*. In each treatment, surface-sterilized roots were used to recover fungal root endophytes deposited in CORFU (Dataset S1). The method is described in *SI Appendix, Materials and Methods*.

Shoot P Measurements by Inductively Coupled Plasma Mass Spectrometry. For determination of shoot P concentration by inductively coupled plasma mass spectrometry (ICP-MS), shoot samples were dried for 2 d at 65 °C before digestion. For mature plants (i.e., ∼3 mo old; GrH, GAR, and WILD experiments), samples were digested using a microwave system (Multiwave 3000; Anton Paar). Approximately 0.3 g of dry homogenized plant material was digested using 4 mL of HNO_3 (66% vol/vol) and 2 mL of H_2O_2 (30% vol/vol). The microwave program included a power ramp of 10 min followed by 30 min at 1,400 W and a final 15 min of cooling down. Final solutions were diluted 1:5 with deionized water before analysis. For young plants (i.e., 1 mo old; MS agar and sterilized soil experiments) plant material was digested using 500 μL of HNO_3 (66%) at 100 °C for 20 min. Final solutions were diluted 1:10 with deionized water before analysis. Solution blanks were included. The P concentration was determined using an Agilent 7700 ICP-MS (Agilent Technologies) following the manufacturer's instructions.

Fungal Microbiome Analysis. For fungal community description, DNA was extracted from each compartment, i.e., root, rhizosphere, and bulk soil, and used for fungal ITS2 PCR amplification with primers ITS9/ITS4 (*SI Appendix*, Table S4). Tagged amplicons were sequenced using an Illumina Miseq platform producing 2 × 300 paired-end reads, and data analysis was conducted in Mothur (48). The final 3′388.918 high-quality fungal reads were clustered using de novo OTU picking at 97% sequence similarity. After discarding low abundance (<50 reads) and nonfungal OTUs, 2.966 OTUs were obtained, and each OTU was taxonomically classified using the UNITE database in Mothur. On average, 38.405 final fungal reads and 567 OTUs were obtained per sample (*SI Appendix*, Table S5). Highly conserved root OTUs (>85% prevalence across all root samples) are given in Dataset S4. The detailed procedure is given in *SI Appendix, Materials and Methods*. The raw sequencing data have been deposited at the National Center for Biotechnology Information (NCBI) Short Read Archive under Bioproject PRJNA386947.

Statistical Methods Used for Microbiome Studies. Analyses were conducted in R 3.2.3. The OTU relative abundances were calculated and transformed using a $\log_{10}(x + 1)$ formula. Bray–Curtis dissimilarities between samples were calculated using the "vegdist" function of the vegan package (49) and used for principal coordinates analysis using the "dudi.pco" function of the ADE4 package (50). Fungal alpha diversity was estimated in each sample using the Shannon diversity index (H) calculated in Mothur. Means were compared with ANOVA followed by Tukey's HSD ($P < 0.05$). PERMANOVA on Bray–Curtis dissimilarities was conducted to study the effect of different factors on the structure of fungal communities using the "Adonis" function of the vegan package (at $P < 0.05$). As previously performed in a study on metal bioaccumulation in plants (51), we calculated a P-accumulation factor (P concentration in the plant shoot divided by the plant-available P concentration in the soil). Plant-available P in the soil (*SI Appendix*, Table S2) was measured using the ammonium-acetate EDTA extraction method (AAE10) by the Laboratory for Soil Analysis (Thun, Switzerland), and shoot P was measured by ICP-MS as indicated above.

Effect of F229 Inoculation on *A. alpina* Growth in Sterile-Soil Microcosms. For sterile-soil microcosms, 250 g of soil Gal (low plant-available P: 3.7 mg/kg, *SI Appendix*, Table S2) was put into 500-mL glass jars (Weck) and autoclaved twice with a 48-h interval. Since fungus F229 did not sporulate under our experimental conditions, a mycelium suspension was used for inoculation. After growing the fungus for 4 wk on malt yeast peptone agar, the fungal mycelium was recovered from the surface of the agar, weighted, and diluted to 250 mg/mL with sterile water, and ∼30 glass beads per milliliter (Ø 1.7–2.1 mm) (Carl Roth) were added before grinding twice at 6,200 × *g* for 10 s in a Precellys instrument (Bertin Technologies). The mycelium was subsequently washed twice through addition of nine volumes of water and centrifugation at 700 × *g* for 2 min. The final pellet was resuspended in sterile water, and the mycelial concentration was adjusted to 10 mg/mL. Fungus-treated microcosms were inoculated with 10 mL of this inoculation suspension (100 mg of mycelium per pot), heat-killed controls received 10 mL of this suspension after autoclaving, and water controls received 10 mL of sterile water. Plate dilution series were made with the inoculum suspension, and colony counting after 4 d indicated a level of inoculation of $1.32 \pm 0.8 \times 10^4$ propagules per pot. Soil humidity was adjusted to 70% of the water-holding capacity without further watering. *A. alpina* F1gal seeds were surface-sterilized as described in *SI Appendix, Materials and Methods*, and stratified for 1 wk at 4 °C on moist sterile filter paper, and 10 seeds were subsequently placed on the soil surface in each microcosm 2 d after fungal inoculation. The microcosms were closed and placed in a phytochamber (Versatile Environmental Test Chamber; Sanyo) with 16-h/8-h day/night cycles at 22/18 °C and 70% relative humidity. The microcosms were randomized every other day. After 28 d, plants were harvested individually, shoot weight was measured, and all of the shoots from one microcosm were pooled to measure shoot P content by ICP-MS as described above. One root system per microcosm was collected for microscopy analysis of fungal colonization. Microscopy analyses were conducted as described in *SI Appendix, Materials and Methods*. The experiment was repeated four times; three experiments included the "heat-killed"' treatment, with three to five microcosms per treatment. Due to a reduced germination rate, on average seven plants per microcosm could be sampled ($n = 10–39$ per treatment per experiment). Data normality was checked, and means were compared with the Mann–Whitney test ($P < 0.05$). The impact of fungal inoculation on *A. alpina* growth was also studied on MS agar; the detailed procedure is given in *SI Appendix, Materials and Methods*.

^{33}P Translocation Experiments. In vitro hyphal transfer of ^{33}P orthophosphate to the plant by F229 was studied as described in ref. 12. A bicompartment system was established by placing two small round petri plates (Ø 3.8 cm) constituting the HC into a square petri plate that was filled with low-P (100 μM P) or high-P (1,000 μM P) MS agar up to the rim of the small plate, which served as the RHC. Roots were precluded from growing into the HC by regularly moving root tips before root ingrowth, thus maintaining the physical barrier between both compartments (Fig. 3*D*). Four-week-old fungal potato dextrose agar plates were used to inoculate the HC by transferring a 0.5-cm^3 agar plug containing fungal hyphae. *A. alpina* F1gal seeds were surface-sterilized, stratified, and allowed to germinate on low-P MS agar as described in *SI Appendix, Materials and Methods*. After 2 wk of fungal growth in the bicompartment system, two 1-wk-old *A. alpina* F1gal seedlings were transferred to the RHC. No fungus was added to the HC in the mock treatments. Plates were closed with Micropore tape, placed vertically in a phytochamber (Sanyo, 16 h/8 h day/night cycles at 22/18 °C and 70% relative humidity), and incubated for another 2 wk. When fungal hyphae had crossed the physical barrier between both compartments, 350 kBq of carrier-free ^{33}P-labeled H_3PO_4 (∼3 pmol; Hartmann Analytik) was added to the HC. Plates were incubated horizontally in the phytochamber, and after 7 (experiment 1), 10 (experiment 2), or 15 d (experiment 3), plant shoots were sampled, dried overnight at 65 °C, digested with 500 μL 66% HNO_3 at 100 °C for 20 min with addition of 250 μL H_2O_2, and heated at 100 °C for 1 min. The solution was diluted 1:10, and 500 μL was mixed with 4.5 mL of scintillation mixture (Rotiszint eco plus; Roth) and used for detection of ^{33}P signals with a scintillation counter (Beckman Coulter LS 6500). Between 5 and 36 plants were analyzed per treatment per experiment. The effect of Benomyl on fungal ^{33}P translocation across the compartments was studied in low-P (100 μM P) MS agar in the absence of the plant by sampling a 1-cm^2 agar piece from the RHC 7 d after addition of ^{33}P to the HC (*SI Appendix*, Fig. S8*A*). For Benomyl-treated samples, the HC was covered with 1 mL of a Benomyl (Institute of Organic Industrial Chemistry, Warsaw) solution of 3 μg/mL (wt/vol), and plates were left open to dry for 1 h before addition of ^{33}P to the HC. The rest of the experiment was conducted as described above. The experiment was repeated three times with 5–20 replicates.

Phylogenetic Analysis of F229 and Related Ascomycetes. The F229 genome (NCBI BioProject PRJNA378526) was sequenced and annotated as described in *SI Appendix, Materials and Methods*. A multigene phylogenetic analysis was

conducted on 51 ascomycete genomes including F229. A phylogenetic tree was inferred from five housekeeping genes: 28S, 18S, Rpb1, Rpb2, and EF1α. For each gene, the nucleotide sequences retrieved from the genomes were aligned with MUSCLE (28), and informative positions were selected using Gblocks with relaxed parameters (52). Alignments were concatenated and used to compute a maximum-likelihood tree using PhyML (29) with the GTR+I+γ model and the SH-aLRT method for branch-support (1,000 iterations) calculation using Seaview (53).

Comparative Analysis of the Abundance of CAZymes in F229 and Related Ascomycetes. The abundance of CAZymes in the F229 genome was compared with that of 51 other ascomycetes genomes with plant-beneficial, plant-pathogenic, saprophytic, and plant unrelated lifestyles. In each genome, CAZyme modules were identified as described in *SI Appendix, Materials and Methods*, and their abundances were compared (Dataset S3). CAZyme profiles were compared by hierarchical clustering (Euclidean distances with average linkage method) in Morpheus (https://software.broadinstitute.org/morpheus/), and differences in the abundance of particular CAZymes between groups were assessed with *t* tests ($P < 0.01$).

Other experimental procedures are described in *SI Appendix, Materials and Methods*.

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