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Phosphorus Effects on the Mycelium and Storage Structures of an Arbuscular Mycorrhizal Fungus as Studied in the Soil and Roots by Analysis of Fatty Acid Signatures

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The distribution of an arbuscular mycorrhizal (AM) fungus between soil and roots, and between mycelial and storage structures, was studied by use of the fatty acid signature 16:1ω5. Increasing the soil phosphorus level resulted in a decrease in the level of the fatty acid 16:1ω5 in the soil and roots. A similar decrease was detected by microscopic measurements of root colonization and of the length of AM fungal hyphae in the soil. The fatty acid 16:1ω5 was estimated from two types of lipids, phospholipids and neutral lipids, which mainly represent membrane lipids and storage lipids, respectively. The numbers of spores of the AM fungus formed in the soil correlated most closely with neutral lipid fatty acid 16:1ω5, whereas the hyphal length in the soil correlated most closely with phospholipid fatty acid 16:1ω5. The fungal neutral lipid/phospholipid ratio in the extraradical mycelium was positively correlated with the level of root infection and thus decreased with increasing applications of P. The neutral lipid/phospholipid ratio indicated that at high P levels, less carbon was allocated to storage structures. At all levels of P applied, the major part of the AM fungus was found to be present outside the roots, as estimated from phospholipid fatty acid 16:1ω5. The ratio of extraradical biomass/intraradical biomass was not affected by the application of P, except for a decrease at the highest level of P applied.

All fungi that form arbuscular mycorrhiza (AM) are dependent upon colonization of a root system. The fungus then grows outwards from the roots into the surrounding soil to form the extraradical mycelium, which is a key factor in the nutrient dynamics of mycorrhizal symbiosis. Several techniques for assessing the biomass of the extraradical mycelium of AM fungi have been employed; most of them are based on either microscopy by methods similar to those for soil fungi generally (16) or on the weighing of extracted hyphae (34). Since microscopical methods are time-consuming and based on structures that stay intact after death of the fungus, techniques based on biochemical signatures are attractive, particularly if they are suitable for quantifying the living biomass in both the intraradical and the extraradical mycelia. Chitin has been employed as a biochemical signature for extraradical AM mycelium (6, 7), and the use of sterols for this purpose has been examined as well (35). The chitin assay has various drawbacks (36), however, one of them being the high and variable background levels of chitin in soil samples. This makes the technique difficult to apply under natural soil conditions. Another limitation is that chitin decomposes slowly after hyphal death, limiting its suitability for estimating living biomass.

Phospholipid fatty acids (PLFAs) can be used for detecting changes in the microbial community structure in soil (11, 38) since organisms differ in the fatty acids they contain (37). Analyses of fatty acids obtained from lipids of the spores and roots of AM have shown AM fungi to contain several fatty acids in combinations not commonly found in other organisms (4, 19, 25, 27, 28). The fatty acid composition varies between different AM fungi (15, 32), but fatty acid compositions are highly similar for different isolates of a given AM fungal species (5). We have shown recently that the fatty acid signatures of AM fungi (mainly 16:1ω5 and 20:5) can be used to estimate the amount of AM fungal mycelium both in the soil and roots (26). Furthermore, since the analysis of fatty acid signatures can be made for phospholipids (membrane lipids) and for neutral lipids (storage lipids) separately, it can also be used to differentiate between the mycelium and the storage structures in AM fungi.

The generally adverse effect of high soil P levels on AM formation is well documented (1, 3, 18, 20, 23) and is mainly caused by higher P concentrations in the roots (33). It has also been shown that high P levels in the soil can reduce not only spore germination and hyphal growth from the germinated spores (21) but also early colonization of the roots and growth of the extraradical mycelium (22). There are only a few studies, however, concerning the effects of P on the extraradical mycelium of AM fungi, and no attempts have been made to investigate its effects on the root-soil partitioning in these fungi.

In the present study, the effects which an increase in the soil P level has on the occurrence of storage and membrane lipids and on the relative distribution of *Glomus caledonium* between its intra- and extraradical components are examined. The usefulness of fatty acid signatures as biomass indicators for AM fungi is also evaluated through comparisons with conventional methods involving microscopy.

MATERIALS AND METHODS

Experimental design and biological material. Cucumber plants (*Cucumis sativus* L., cv. Aminex, F1 hybrid) were grown in 40-mm-diameter polyvinyl chloride tubes provided with two lateral compartments, one which only hyphae could enter (HC) and the other which both roots and hyphae could enter (RC), as described by Pearson and Jakobsen (29). The HC was separated from the main tube by a 37-μm-pore-size nylon mesh, and the RC was separated from the main tube by a 700-μm-pore-size mesh. AM fungal inoculum was obtained from

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Trifolium subterraneum pot cultures of *G. caledonium* (Nicol. and Gerd.) Trappe and Gerdemann, isolate RIS42 BEG 15.

Two identical experiments were performed by use of a factorial design. Inorganic P was applied at five different rates to plants both with and without the AM fungal inoculum. There were four replicates of each treatment, and each experiment included 40 growth units. Root colonization was lower in the second experiment than in the first experiment. Most of the results presented are from the first experiment.

Growth conditions and harvest. The solid growth medium in the polyvinyl chloride tubes was a 1:1 mixture of sieved (4-mm pore size) moraine clay loam and quartz sand. The clay loam was a topsoil from Tåstrup, Denmark, with properties as described by Jakobsen and Nielsen (17). The soil mixture was irradiated (10 kGy, 10-MeV electron beam) to eliminate indigenous AM fungi. The following nutrients were mixed with the soil (in milligrams per kilogram of dry soil^{-1}): NH_4NO_3, 86; K_2SO_4, 70; $CaCl_2$, 70; $MgSO_4 \cdot 7H_2O$, 20; $MnSO_4 \cdot 7H_2O$, 10; $CuSO_4 \cdot 5H_2O$, 2.2; $ZnSO_4 \cdot 7H_2O$, 5; $CoSO_4 \cdot 7H_2O$, 0.33; $Na_2MoO_4 \cdot 2H_2O$, 0.2. The soil originally contained 8.5 mg of P kg^{-1} (0.5 M $NaHCO_3$ extractable), with additional P added as KH_2PO_4 at 0, 15, 30, 60, and 90 mg of P per kg of soil.

The main tube in each of the growth units contained 390 g of soil; 50 g of this was replaced in the mycorrhizal treatment by the inoculum. The inoculum was placed in the main tube between the two lateral compartments, each of which contained 50 g of soil. To reintroduce a saprophytic microbial community, 40 ml of filtrate (<38-μm pore size), prepared from 100 g of inoculum and 50 g of garden soil suspended in 500 ml of H_2O, was added to the growth tubes. Seven days later, two surface-sterilized cucumber seeds were sown in each tube, and after another 5 days, the seedlings were thinned to one per tube. The plants were supplied with 20 mg of N (as NH_4NO_3) 13 days after sowing and 30 mg of N both 19 and 24 days after sowing, thus with a total of 80 mg N altogether. The pots were watered each day to 60% of their water-holding capacity. The conditions during the growth of the plants were as described by Pearson and Jakobsen (29).

The plants were harvested 34 days after sowing. The shoots and roots from the main compartment were dried and weighed, and the P concentrations were determined as described by Pearson and Jakobsen (29). A subsample of the root in the main compartment was used for estimating the AM colonization. The roots were hand picked from the soil in the RC and divided into two subsamples, one of them being stored at −20°C for later fatty acid measurements and the other being used for AM colonization measurements. Soil samples from both the HC and the RC were carefully homogenized and then stored at −20°C for fatty acid measurements or dried for measurements of hyphal length.

Visual quantification of root colonization, numbers of spores, and length of AM hyphae. A line intercept method was used to assess root length and root colonization after weighed root samples had been cleared in KOH and stained with trypan blue. The presence or absence of mycorrhizal colonization at each intercept was used to determine the percentage of colonization and the colonized root length.

The length of the hyphae was measured by an aqueous extraction and membrane filter-grid intercept method modified after that of Abbott et al. (1). The modifications included the use of 3-ml aliquots and 20-mm-diameter filters. Duplicate filters were prepared from each of the duplicate 2-g soil samples of both the HC and the RC, the hyphae being counted in 25 random viewing fields per filter at ×200 magnification. The measurements of hyphal length for the mycorrhizal treatments were corrected for background levels as measured in the nonmycorrhizal (NM) plants.

Spore counts were performed by wet sieving with 50-μm-pore-size mesh (14). The spores were counted under a compound microscope at ×50 magnification.

Analysis of neutral lipid fatty acids (NLFAs) and PLFAs. Lipid extraction followed the method of Frostegård et al. (12), which is a modification of the Bligh and Dyer (8) procedure. Freeze-dried and ground root samples (30 mg) as well as soil samples (3 g) were extracted in 10 ml of one-phase chloroform-methanol-citrate (1:2:0.8 [vol/vol/vol]; pH 4.0) buffer. After centrifugation, the pellets were washed with 5 ml of the one-phase mixture, and the supernatants were combined. The extract was split into two phases by adding 4 ml of chloroform and 4 ml of citrate buffer. The extracted lipids were fractionated on silicic acid (100/200 mesh; Unisil) columns into neutral, intermediate, and polar lipids by elution with 5 ml of chloroform, 20 ml of acetone, and 5 ml of methanol, respectively. The neutral lipids (mainly storage lipids) and polar lipids (containing phospholipids) were dried under nitrogen, with methyl nonadecanoate (fatty acid methyl ester 19:0) added then as an internal standard (23 μg ml^{-1}). The samples were subjected to mild alkaline methanolysis (10), which transformed the fatty acids in the neutral lipids and the phospholipids into free fatty acid methyl esters. These were analyzed on a Hewlett-Packard 5890 gas chromatograph equipped with a flame ionization detector and a 50-m HP5 capillary column as described by Frostegård et al. (13).

Identification of the fatty acids was achieved by use of the relative retention times, i.e., in comparison to that of the internal standard. These were compared to those identified earlier by gas chromatography-mass spectrometry. The nomenclature of the fatty acids follows that used by Tunlid and White (37).

Statistics. The effects of the mycorrhizal treatment on the plant parameters were tested by analysis of variance (F-ratio), and the relationship between the different fungal parameters was assessed by linear regression.

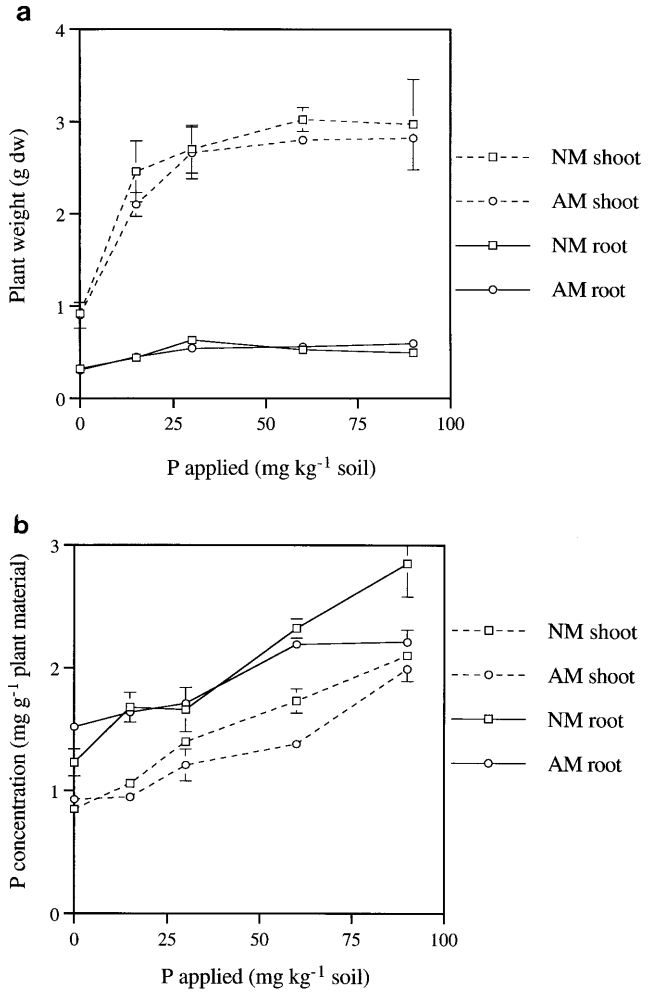

FIG. 1. Dry weights (dw) (a) and P concentrations (b) in shoots and roots of cucumber plants as influenced by applications of P. The plants were either colonized by *G. caledonium* (AM) or were left uninoculated (NM). Error bars represent standard errors. (n = 4)

RESULTS

Plant growth and P content. The shoot biomass of the cucumber increased markedly in response to the P being applied (Fig. 1a). The root biomass also increased but to a lesser extent. The growth responses to the application of P were similar for the mycorrhizal and NM plants. Application of P resulted in an increase in P concentration in both the shoots and the roots (Fig. 1b). In comparison with the NM plants, the AM-colonized plants demonstrated a decrease in P concentration in the shoots ($P < 0.01$) but not in the roots.

Root length and microscopic measurements of AM structures. The percent of root colonization decreased as the application of P increased. The colonization of the roots in the main tube decreased from 84 to 27%, with a corresponding decrease in the RC of from 73 to 3% (Fig. 2a). The total root length in the RC increased with the application of 15, 30, and 60 mg of P kg of soil^{-1} but decreased again at the highest level of P applied (Fig. 2b). The total root length was generally shorter in the mycorrhizal than in the NM plants (Fig. 2b). The mycorrhizal root length was not affected by the application of P up to 60 mg kg^{-1}, whereas at 90 mg of P kg^{-1}, it tended to decrease (Fig. 2b).

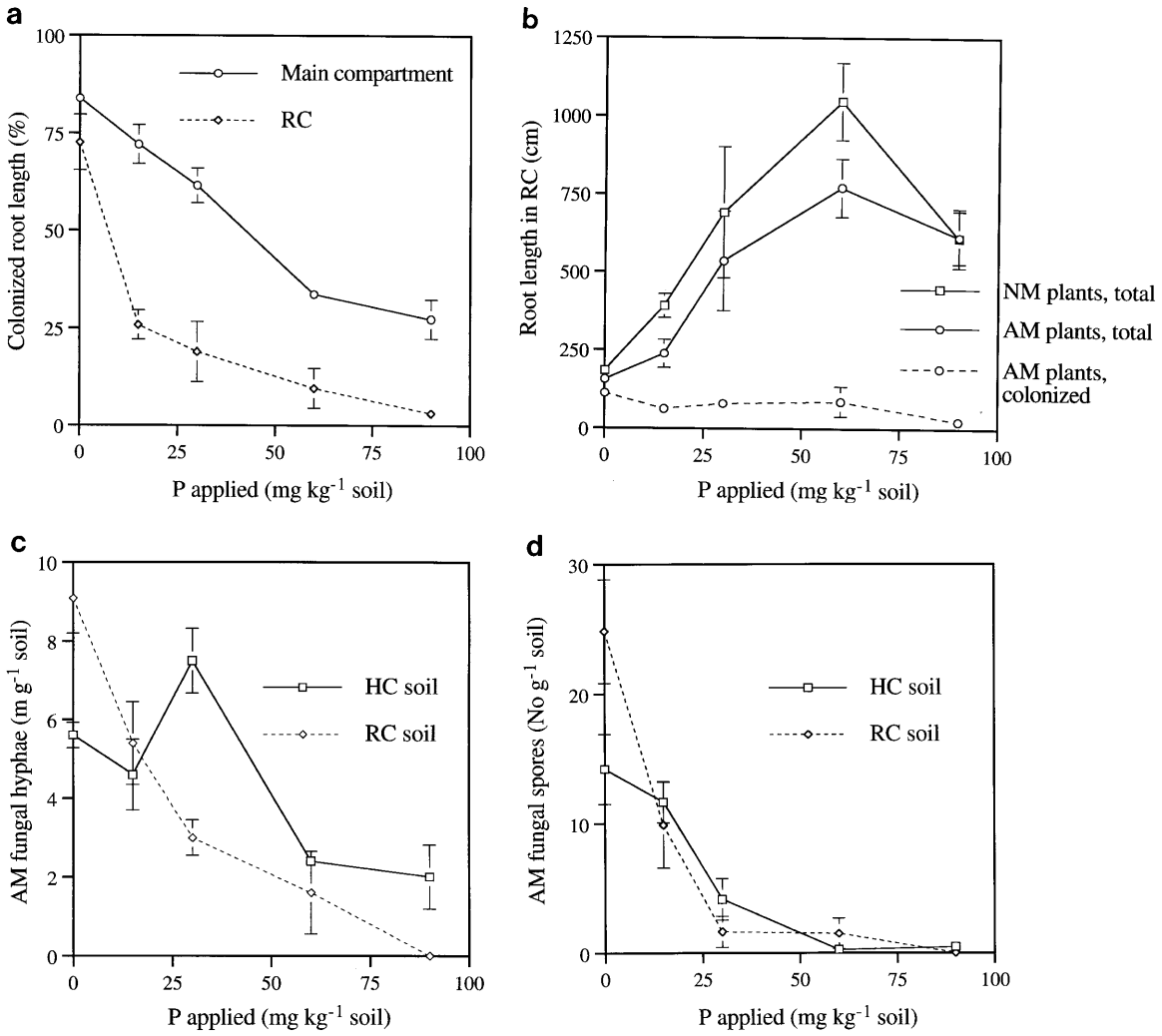

FIG. 2. Microscopical measurements of the percentage of colonized root length (a), the total and colonized root length in RC in AM cucumber plants (b), the length of AM fungal hyphae in soil (c), and the numbers of AM fungal spores (d) as influenced by applications of P to the soil (n = 4; error bars represent standard errors).

The hyphal length was greater in the RC than in the HC soil when no P had been applied, but it decreased to nil in the RC at the highest P level (Fig. 2c); the greatest hyphal length in the root-free HC soil was observed at 30 mg of P kg^{-1}. In contrast with hyphal growth in the RC, hyphal growth was detected in the HC at the highest P level. The spore numbers were higher in the HC than in the RC soil when no P had been applied but decreased to rather low levels that were similar for both at 30 mg of P kg^{-1} (Fig. 2d).

Fatty acid signatures. PLFA 16:1ω5 and NLFA 16:1ω5 were analyzed. The application of P affected the levels of this fatty acid signature of *G. caledonium* in both the soil and the roots. The NM values of PLFA 16:1ω5 were between 0.45 and 0.58 nmol g of $soil^{-1}$, whereas 16:1ω5 was not detectable in the NM roots. Neither PLFA nor NLFA 20:5 was detected in any of the root or soil samples from the NM treatments. The values for NLFA 16:1ω5 in NM soils were similar to those for PLFA 16:1ω5. Since the content of 16:1ω5 and 20:5 in the NM controls was not affected by the application of P, mean background values could be calculated. These values were subtracted from the values for the AM treatments shown in Fig. 3 to 6.

Both PLFA and NLFA 16:1ω5 from the AM-inoculated treatments decreased in the roots, the RC soil, and the HC soil with an increase in the application of P (Fig. 3a and b). A marked decrease in PLFA and NLFA 16:1ω5 was observed even at the lowest level of P applied (15 mg of P kg^{-1}), except in the case of the HC soil, where this level of P gave the highest level of PLFA 16:1ω5. In accordance with the measurements of hyphal length and spore numbers, both PLFA and NLFA 16:1ω5 were higher in the RC than in the HC when no P had been applied (the amounts being equal to or lower than this in the RC) (Fig. 3a and b).

Both PLFA and NLFA 20:5 showed patterns similar to that for 16:1ω5 (Fig. 3c and d), although at the highest level of P applied, fatty acid 20:5 could not be detected in the AM treatments.

Fatty acid 16:0 is present in AM fungi, but in contrast to the signature fatty acid 16:1ω5, it is also common in most other organisms, including plants. Only NLFA 16:0 increased in response to AM, the pattern of increase being similar to that observed for NLFA 16:1ω5 (data not shown).

Less colonization of the root in the main tube (which was not sampled for soil analyses) was found in a second experiment (between 13 and 39%); little mycelium grew out into the lateral compartments (HC and RC), and the roots in the RC were only sparsely colonized (between 0 and 19%). A difference in

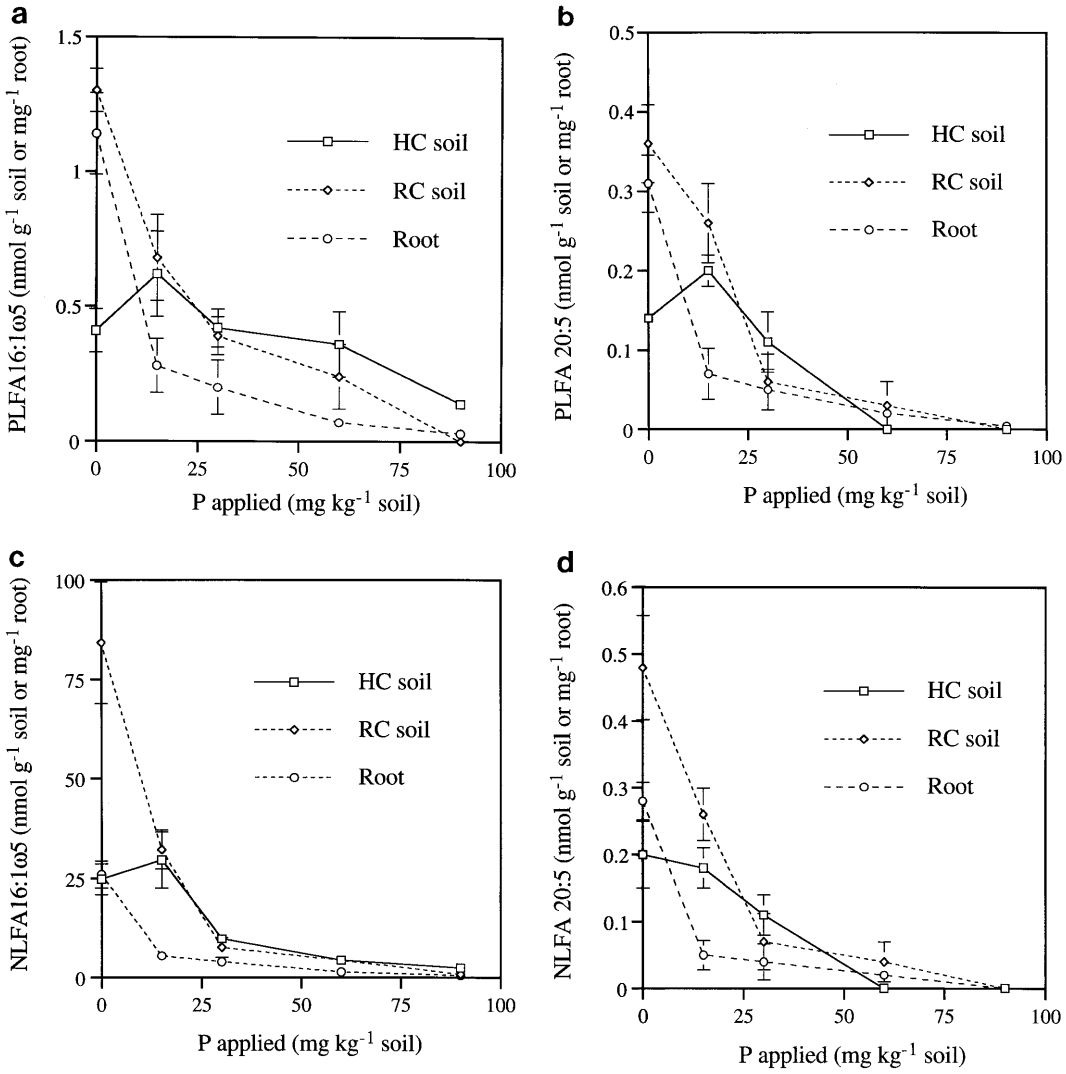

FIG. 3. Response of AM fungi in the soil and roots to applications of P as measured by PLFAs 16:1ω5 (a) and 20:5 (b) and by NLFAs 16:1ω5 (c) and 20:5 (d). The values for the NM growth units have been subtracted ($n = 4$; error bars represent standard errors).

the amounts of PLFA and NLFA 16:1ω5 compared with the NM controls was observed in this experiment as well but only for the soil samples to which 0 or 15 mg of P kg^{-1} was applied. Colonization could be detected, however, by the presence of fatty acid 16:1ω5 in the roots for all P treatments except for the application of 90 mg of P kg^{-1}. Effects similar to those for the application of P in the first experiment were found.

Comparisons between microscopical measurements and fatty acid signatures. PLFA and NLFA 16:1ω5 were found to correlate ($P < 0.001$) with spore counts and measurements of hyphal length (Fig. 4a to d). NLFA 16:1ω5 was more strongly correlated than PLFA 16:1ω5 with the numbers of spores in the soil. The correlation between PLFA 16:1ω5 and the number of spores (Fig. 4a) also demonstrates that in some cases, high values for PLFA 16:1ω5 were obtained when no spores or only a few of them were formed. Accordingly, as shown in Fig. 4d, rather large hyphal lengths can be detected even when only small amounts of NLFA 16:1ω5 are present, whereas the regression of the hyphal length on PLFA 16:1ω5 passes almost through the origin. Thus, the amount of PLFA 16:1ω5 appeared to be a good indicator of hyphal length in the AM fungus, whereas the amount of NLFA 16:1ω5 was an indicator of the number of spores formed in the soil.

The AM fungal colonization of the roots in the RC correlated well with the content both of PLFA 16:1ω5 (Fig. 4e) and of NLFA 16:1ω5 (Fig. 4f) in the roots. Thus, the analysis of the fatty acid 16:1ω5 and the traditional methods showed similar decreases in AM when P was applied.

Neutral lipid/phospholipid ratios. The ratio of neutral lipids to phospholipids in the AM mycelium can be estimated by the use of fatty acid 16:1ω5, assuming the proportions of this fatty acid in the two lipid classes to be similar at each application of P. The neutral lipid/phospholipid ratio showed a dramatic decrease with the application of 15 mg of P kg^{-1} (Fig. 5a). This was especially evident in the soil, whereas only a slight decrease in the neutral lipid/phospholipid ratio was observed for the root samples. A positive correlation ($P < 0.001$) between the neutral lipid/phospholipid ratio in the soil and root colonization was found (Fig. 5b), indicating that at high colonization levels, the fungus allocated proportionally more carbon to storage structures than it did at low colonization levels. In accordance with this, a positive correlation was found between

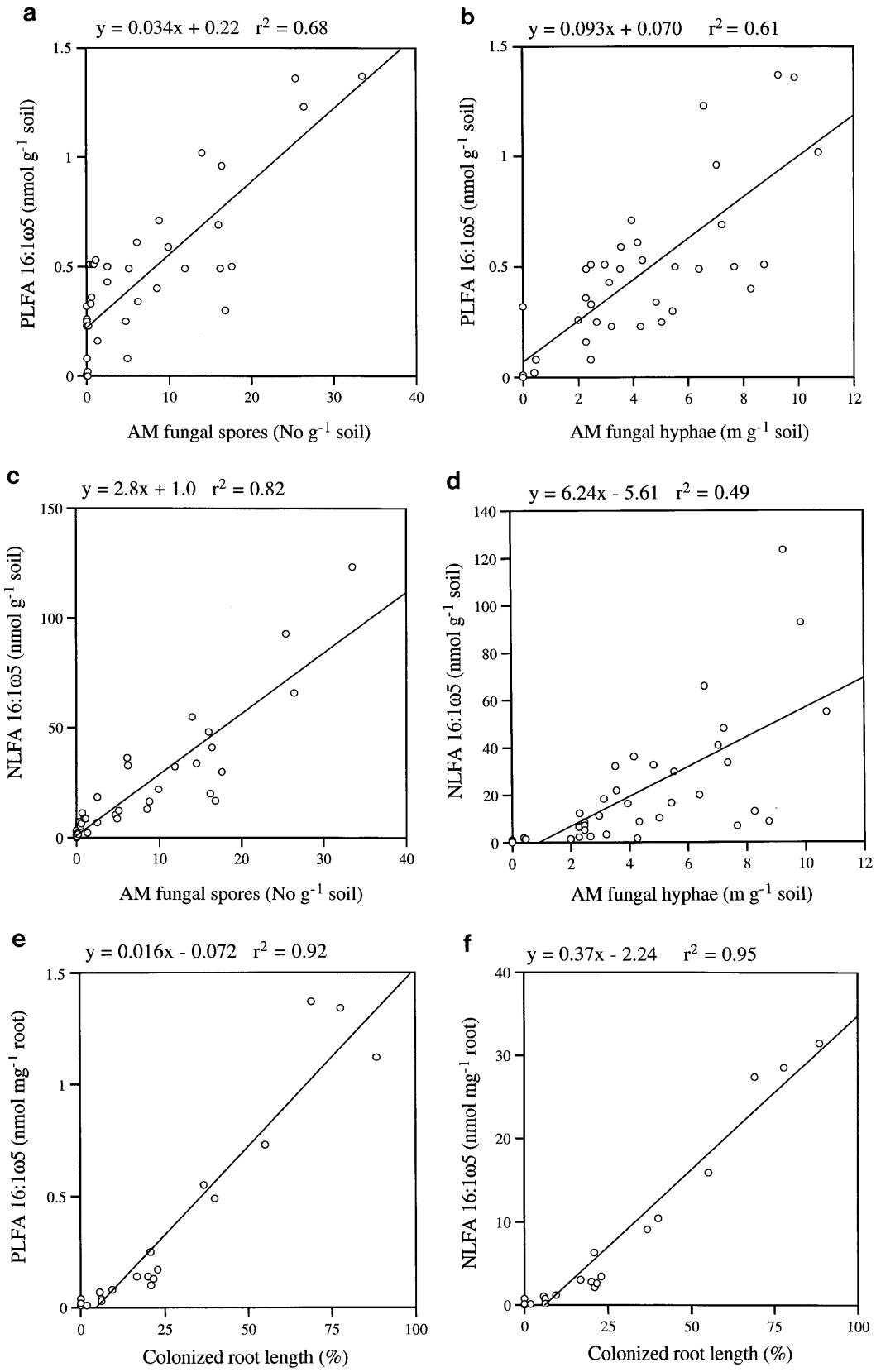

FIG. 4. (a to d) Linear regression between the AM fungal fatty acid signature 16:1ω5 and microscopical measurements of the spores and the external mycelium (n = 40); (e and f) linear regression between the fatty acid 16:1ω5 in the roots and root colonization (n = 20).

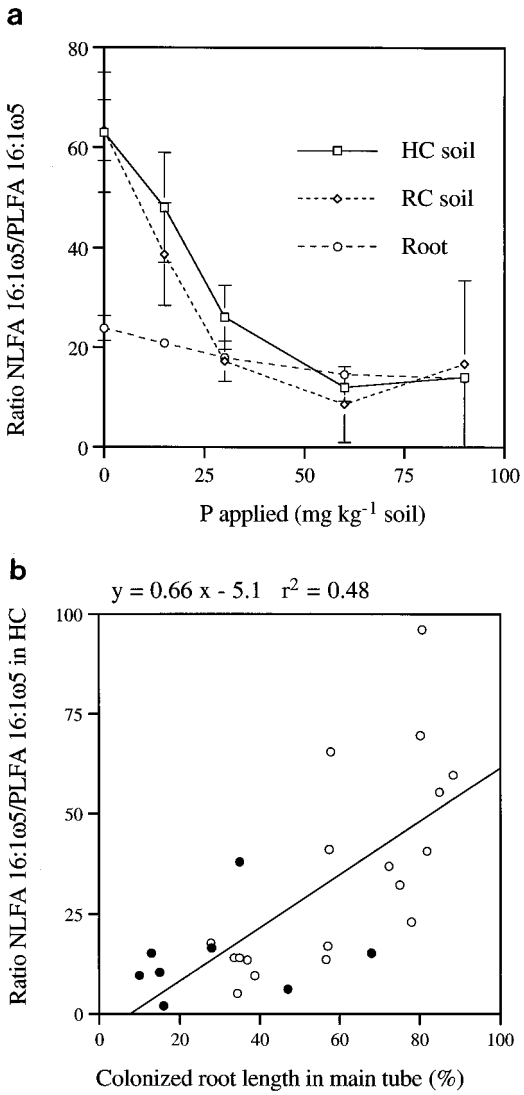

FIG. 5. The neutral lipid/phospholipid ratio in *G. caledonium*, estimated with the fatty acid signature 16:1ω5, as influenced by applications of P (a) and examined in relation to root colonization in two separate experiments (b). Open and closed circles in panel b denote the values obtained in the two identical experiments.

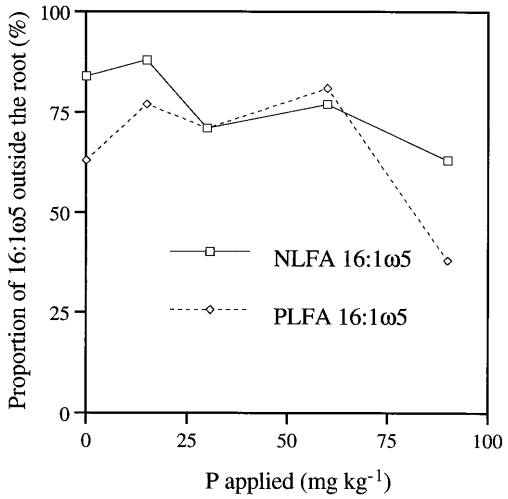

FIG. 6. Calculated proportion of the fatty acid 16:1ω5 in the AM fungal mycelium found outside the roots with different applications of P to the soil.

the neutral lipid/phospholipid ratio and the number of spores per unit length of the hyphae ($r^2 = 0.48$, $P < 0.001$).

Intra- and extraradical distribution. The present study was not directly designed for calculating the total intra- and extraradical AM fungal biomass, since only the extraradical AM hyphae in the lateral compartments that were with and without roots were measured and not that in the main tube. Figure 6 shows the estimates obtained of the percentage of extraradical AM fungal biomass in the total growth unit. The estimates were based on PLFA and NLFA 16:1ω5, assuming the amount of AM fungi in the soil in the main tube and in the soil in the lateral RC to be the same. Our calculations indicate that the major part of the AM fungus was present as extraradical mycelium.

DISCUSSION

This study demonstrates that the effects on the intra- and extraradical growth of an AM fungus of increasing the soil P levels can be assessed by measuring the content of the fatty acids 16:1ω5 and 20:5 in the soil and in the roots. These fatty acid signatures correlated well with the measurements of fungal growth obtained by microscopy, and the methods are therefore complementary. Although direct microscopy is the tool used to confirm the presence and the identity of fungal structures in the roots and the soil, the fatty acid signatures provide certain further information. They reflect the living biomass of the AM fungi and can easily be used to determine the biomass distribution between the intra- and extraradical phases of AM fungi.

The phospholipids are supposed to degrade rapidly after the death of organisms (37); however, the microscopical methods used here are based on the detection of cell walls, which often stay intact in the soil long after the death of the hyphae (36). Furthermore, microscopical estimates of AM fungi seldom take into account the thickness of the hyphae or the specific intensity of root colonization present. Both PLFA and NLFA 16:1ω5 in the roots correlated closely with root colonization as estimated microscopically (Fig. 4e and f). Examining these relationships reveals that at lower colonization levels, there is a tendency for the fungal biomass to be overestimated by the microscopical method but not by the use of the fatty acid signature. Low percentages of root colonization are typically accompanied by a low colonization intensity, as reflected in the amounts of PLFA and NLFA 16:1ω5 but not in the percentage of root length that is colonized.

The negative effect of application of P on AM formation is well known and has been demonstrated repeatedly by traditional microscopical methods. Peng et al. (30) have reported previously that in AM *Citrus* plants, the application of P to the soil reduces the total content of fatty acids that originates from the fungus in the roots. However, the authors did not distinguish between PLFAs and NLFAs. It is likely that the fatty acid content obtained by Peng et al. (30) is equivalent to our NLFAs, since this fraction represents the main portion of the lipid content in AM fungi. With the roots, we obtained about 20 times as high a concentration of NLFA 16:1ω5 as that of PLFA 16:1ω5 (Fig. 5a).

The close correlation between AM fungal NLFA 16:1ω5 and the numbers of AM fungal spores (Fig. 4c) indicates that the spores are the main neutral lipid-containing structures in *G.*

caledonium. Cooper and Lösel (9) showed that spores contained neutral lipids in the form of triglyceride, diglyceride, and free fatty acids, and lipids were also observed in the hyphae as oil droplets. In the present study, we estimated that each spore contained 2 to 5 nmol of NLFA 16:1ω5. The close correlation between PLFA 16:1ω5 and the length of the mycorrhizal hyphae in the soil (Fig. 4b) fits well with the hypothesis that the amount of phospholipids provides an indication of the membrane area (37), which should be rather constant for a given length of the hyphae. PLFAs can thus be considered to be a biomass indicator for the hyphae. Olsson et al. (26) estimated a content of 0.2 nmol of PLFA 16:1ω5 per m of hyphae in *G. caledonium*. The corresponding estimate in this study is 0.1 nmol per m of hyphae. The lower figure obtained in the present study could be due to the inclusion of hyphae of thinner diameter in the length measurements, a matter reflected in the higher values for hyphal length obtained here. In contrast to the phospholipids, the neutral lipids can vary with the length of the hyphae due to spore formation and/or to the nutritional status of the mycelium.

The conclusion that the PLFA and NLFA measurements reflect different structures in AM fungi is supported by estimates of the biovolume of the hyphae and the spores. The estimates were based on an assumed mean diameter of the hyphae of 3 μm and an assumed mean diameter of the spores of 150 μm and indicating that, at low applications of P, about 50% of the extraradical biovolume was present as spores. Similar calculations of the total membrane area indicated the spores to represent less than 2% of the total mycelial surface area. Thus, it seems likely that the amount of PLFAs, which represents the membrane lipids, correlates with the mycelial length. Since in comparison with hyphae, AM fungal spores are rich in neutral lipids, a positive correlation between the numbers of spores and NLFA 16:1ω5 could be expected.

The neutral lipid/phospholipid ratio in the extraradical mycelium correlated positively with root colonization in the main tube. Thus, when conditions became less suitable for AM fungal colonization of the root, the fungus invested proportionally less in storage lipids, that is, in spores. One interpretation of this could be that at higher applications of P, when less carbon is allocated to the roots, there is less of a surplus of carbohydrates that can be used by the fungus for storage structures. However, since we did not monitor AM fungal growth over time, the results could also be conceived as indicating that at high applications of P, there is a delay in sporulation due to the carbon flux from the plant being reduced. For the intraradical AM mycelium of *Glomus fasciculatum*, Abbott et al. (1) observed that vesicle formation (fungal storage structures in the root) was more sensitive to P applications than the amount of intraradical hyphae was. The use of the neutral lipid/phospholipid ratio for estimating carbon allocation to the storage structures of the fungus provides a new AM fungal parameter, one which is not directly available when traditional microscopical methods are employed.

The growth of the extraradical AM mycelium was found to be more negatively influenced in the RC than in the HC by the application of P (Fig. 2c and 3a). This was evident in both experiments and indicates that at high nutrient levels, conditions are less favorable for the growth of the extraradical mycelium near the roots. The differences between the HC and the RC were probably even greater than the measurements indicated, since the soil mycelium in the RC represents both what has grown in from the roots in the main tube and the extraradical mycelium originating from the roots in the RC. The mycelium in the HC originates only from the roots in the main tube. Thus, much less mycelium from the roots in the main tube grew into the RC than into the root-free HC. It is possible that these differences in hyphal growth into the HC and the RC were caused by differences in soil moisture levels. At high root length densities, moisture levels were observed to be maintained at a more constant and adequate level in the HC than in the RC soil.

The utilization of fatty acid signatures as biomass indicators allowed the amounts of AM fungus inside and outside the root to be estimated by use of one method only. Bethlenfalvay et al. (7), using chitin measurements, reported 88% extraradical biomass after 4 weeks of growth in a *Glomus fasciculatum*-soybean symbiosis, but the proportion of extraradical fungal biomass decreased with plant age. Our estimates suggest that increasing P levels in the soil had a limited effect on the extraradical/intraradical biomass ratio, except for the extraradical hyphae tending to contribute a lower proportion of the biomass at the highest level of P applied (90 mg of P kg^{-1}). Abbott et al. (1) found that the length of *G. fasciculatum* hyphae in the soil per length of infected root was reduced at applications of P of 50 mg kg^{-1} and higher. In ectomycorrhiza, there is also a trend towards a decrease in the production of extraradical mycelium in nutrient-rich soils (31). Arnebrant (2) has shown that the formation of extraradical mycelium by ectomycorrhizal fungi is sensitive to applications of N.

We have shown previously that mycelial growth in *G. caledonium* and *Glomus invermaium* increases the PLFAs 16:1ω5, 18:1ω7, 20:4, and 20:5 in the soil (26). In the present study, we used primarily the AM fatty acid signature 16:1ω5 as an indicator of the AM fungus. Also, 20:5 proved to be suitable, but when the soil was treated with high levels of P, it was not detected. Still, the measurement of 20:5 is useful as a control for showing that changes in 16:1ω5 are due to an AM fungus, since the occurrence of significant amounts of both 16:1ω5 and 20:5 in an organism is a rarity (see references 24 and 37).

Since the fatty acid composition can differ with the species of AM fungus involved (15), the best fatty acid for estimating fungal biomass may depend upon the fungus being studied. Such differences between various AM fungi can be a drawback when fatty acid signatures are used for studies conducted with natural soils. However, these differences also provide the possibility for studying interactions between different AM fungal species if they differ in their fatty acid composition as suggested by Graham et al. (15). Since the present study shows that the fatty acid signatures of AM fungi facilitate the study of AM fungal growth under differing soil conditions, we also propose that fatty acid signatures are suitable for studying interactions with other soil organisms. To date, this can be said to hold true only for experimental systems such as pot cultures. However, we have recently used fatty acid signatures for estimating the AM fungal mycelium both in sand dunes and in arable soils.

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