

Do arbuscular mycorrhizal fungi recover from soil disturbance differently?

MIRANDA M. HART* & RICHARD J. READER

Department of Botany, University of Guelph, Guelph, ON Canada, N1G 2W1

Abstract: Disturbance plays a large role in shaping ecological communities. Soil communities in particular are often severely affected by disturbance. However, there is little evidence which describes how these communities respond to disturbance. Arbuscular mycorrhizal (AM) fungi are an example of an important soil constituent. Past studies show that arbuscular mycorrhizal fungi are vulnerable to soil disturbance. This could affect terrestrial communities in many ways as AM fungi have been shown to be important for ecosystem functioning and plant community structure. We suspected that AM fungi differ in their response to soil disturbance due to inherent differences among AM fungal suborders in life history traits. Specifically, we predicted that AM fungi in the suborder Gigasporineae, which colonize roots primarily by spores would be more resilient to soil disturbance than AM fungi in the suborder Glomineae, which colonize roots primarily from hyphal fragments. We also predicted that the growth of AM fungal host plants would be greater when associated with AM fungi that were less compromised by soil disturbance. To test these predictions, we compared the response of AM fungal isolates from the two suborders to soil disturbance, relative to their response in non-disturbed controls in a glass-house pot experiment. AM fungal response was measured in terms of % colonization, root fungal biomass, soil hyphal length, soil ergosterol and spore density. Four different host plants were used and their growth was measured in terms of total plant biomass and % foliar phosphorus. Gigasporineae isolates were significantly less affected than Glomineae isolates by soil disturbance in terms of root colonization, soil colonization and spore densities. Host biomass was positively correlated with AM fungal response. In contrast, no difference in host foliar phosphorus content was detected between hosts grown with Gigasporineae versus Glomineae isolates. These results confirm that AM fungi differ in their response to soil disturbance. Therefore, the composition of the AM fungal community will determine the actual effect of soil disturbance on AM fungal-mediated plant growth.

Resumen: El disturbio desempeña un papel importante en la configuración de las comunidades ecológicas. En particular, con frecuencia las comunidades del suelo se ven afectadas severamente por disturbios. Sin embargo, existe poca evidencia que describa cómo responden estas comunidades al disturbio. Los hongos micorrícicos arbusculares (MA) ejemplifican un importante constituyente del suelo. Estudios pasados muestran que los hongos micorrícicos arbusculares son vulnerables a la perturbación del suelo. Esto podría afectar a las comunidades terrestres de muchas maneras, ya que se ha mostrado que los hongos MA son importantes para el funcionamiento del ecosistema y la estructura de la comunidad vegetal. Nosotros sospechamos que los hongos MA difieren en su respuesta a la perturbación del suelo debido a diferencias inherentes entre los subórdenes de hongos MA relacionadas con sus rasgos de historia de vida. Específicamente, hicimos la predicción de que los hongos MA del suborden Gigasporineae, que colonizan las raíces principalmente por medio de esporas, tendrían una mayor capacidad

*Corresponding Author; e-mail: mhart@uoguelph.ca. Tel: (+1)519-824-4120 x6007. Fax: (+1)519-767-1991

de recuperación a la perturbación del suelo que los hongos MA del suborden Glomineae, los cuales colonizan a las raíces principalmente a partir de fragmentos hifales. También predijimos que el crecimiento de plantas hospederas de hongos MA sería mayor cuando éstas estuvieran asociadas a hongos MA menos comprometidos con la perturbación del suelo. Para poner a prueba estas predicciones, comparamos la respuesta de muestras aisladas de hongos MA de los dos subórdenes a la perturbación del suelo, en relación con su respuesta en controles sin disturbio en un experimento de macetas en invernadero. La respuesta de los hongos AM fue medida en términos del porcentaje de colonización, la biomasa fúngica de las raíces, la longitud de hifas en el suelo, el ergosterol del suelo y la densidad de esporas. Se utilizaron cuatro diferentes plantas hospederas y su crecimiento fue medido en términos de la biomasa vegetal total y el porcentaje de fósforo foliar. Las muestras aisladas de Gigasporineae fueron afectadas por la perturbación del suelo significativamente menos que las de Glomineae, en términos de la colonización de las raíces, la colonización del suelo y las densidades de esporas. La biomasa de los hospederos estuvo correlacionada positivamente con la respuesta de los hongos MA. Por el contrario, no se detectaron diferencias en el contenido de fósforo foliar del hospedero entre hospederos que crecieron con las muestras aisladas de Gigasporineae *versus* las muestras aisladas de Glomineae. Estos resultados confirman que los hongos MA difieren en su respuesta a la perturbación del suelo. Por lo tanto, la composición de la comunidad de hongos MA determinará el efecto real de la perturbación del suelo sobre el crecimiento de las plantas mediado por los hongos MA.

Resumo: O distúrbio joga um largo papel na configuração das comunidades ecológicas. As comunidades do solo, em particular, são frequentemente afectadas severamente pelos distúrbios. Contudo, há pouca evidência que descreva como estas comunidades respondem aos distúrbios. Os fungos micorrízicos arbusculares (AM) são um exemplo de um constituinte importante do solo. Estudos anteriores mostraram que os fungos arbusculares micorrízicos são vulneráveis aos distúrbios do solo. Isto pode afectar as comunidades terrestres de muitas formas já que os fungos arbusculares mostraram ser importantes para o funcionamento do ecossistema e para a estrutura das comunidades vegetais. Suspeita-se que os fungos arbusculares diferem na sua resposta aos distúrbios do solo devido às diferenças inerentes entre as subordens dos fungos arbusculares nas características da história da vida. Predizemos, especialmente, que os fungos AM da subordem Gigasporineae, que colonizam as raízes primariamente por esporos, serão mais resilientes aos distúrbios do solo do que os fungos AM da subordem Glomineae, que colonizam as raízes primariamente através de fragmentos de hifas. Igualmente predizemos que o crescimento das plantas hospedeiras de fungos AM será maior quando associadas com fungos AM que sejam menos afectados pelos distúrbios do solo. Para testar estas predições, comparou-se a resposta dos isolados de fungos AM de duas subordens aos distúrbios do solo em relação à sua resposta em controlos não perturbados em experiência em vaso em estufa. A resposta dos fungos AM foi medida em termos da % de colonização, da biomassa fúngica radicular, comprimento das hifas no solo, ergosterol no solo e densidade dos esporos. Usaram-se quatro diferentes plantas hospedeiras onde o seu crescimento foi medido em termos da biomassa vegetal total e a % do fósforo foliar. Isolados de Gigasporineae foram significativamente menos afectados do que os isolados de Glomineae pelo distúrbio do solo em termos de colonização das raízes, a colonização do solo e densidade dos esporos. A biomassa dos hospedeiros estava positivamente correlacionada com a resposta dos fungos AM. Em contraste, não se detectaram diferenças no teor do fósforo nos isolados do hospedeiro entre hospedeiros crescendo com Gigasporineae *versus* Glomineae. Estes resultados confirmam que os fungos AM diferem na sua resposta aos distúrbios do solo. Assim, a composição da comunidade de fungos AM determinará o efeito actual dos distúrbios do solo no crescimento das plantas mediadas pelos fungos AM.

Key words: Arbuscular mycorrhizal fungi, colonization strategies, gigasporineae, glomineae, soil disturbance.

Introduction

Soil disturbance in tropical systems is a common occurrence due to both natural (e.g. tree falls) and increasingly, human factors such as the conversion of forests into agricultural lands (Allen *et al.* 1996; Janos 1996). The effects of this disturbance on soil populations is poorly understood which may have serious implications for both the re-establishment of natural forests and the viability of agrosystems. Because tropical soils are extremely nutrient poor, the role of soil microbes is essential for the formation and maintenance of viable plant communities (Janos 1980; Lois & Lim 1987; Sieverding 1991). As a result, most tropical plants including tropical crops (e.g. coffee, sugar, tea, citrus, palm and cocoa) are dependent on soil organisms such as AM fungi for survival (Janos 1980; Sieverding 1991).

Arbuscular mycorrhizas are a mutualistic association between glomalean fungi (Division Zygomycota, Order Glomales) and most vascular plants (Smith & Read 1997) which are particularly prone to soil disturbance. With few exceptions, most studies looking at the effect of soil disturbance on AM fungi have not addressed the unique situation of tropical systems. In the studies that exist, the research focused primarily on spore counts (Cuenca *et al.* 1998; Picone 2000) or else did not differentiate the effect of disturbance on different species of AM fungi in the roots (Allen 1998). Jasper *et al.* (1991) looked at the role of disturbance on different genera of AM fungi in the Jarrah forest in Australia and found there was no significant difference in how they responded to disturbance. In general, more studies looking at local AM fungal populations in tropical systems are required before any trends can be established.

In temperate systems, there is considerable evidence that soil disturbance negatively affects AM fungal growth by disrupting the extra-radicle mycelium (Evans & Miller 1990; Jasper *et al.* 1989; McGonigle & Miller 1996). This severs the AM fungal connection to host carbon reserves and decreases its ability to infect new hosts (Jasper *et al.* 1989). As a result of a disrupted AM fungal extra-radicle mycelia, plants associated with AM fungi are also negatively affected following disturbance. For example, plants infected with AM fungi could suffer reduced uptake of mineral nutrients, such

as phosphorus, since disturbance would destroy the absorptive hyphae of AM fungi. In addition, host plants may experience reduced biomass when AM fungi are still able to access host carbon through intact intra-radicle structures. Such decreases in nutrient status and biomass following soil disturbance have been documented for crop plants associated with AM fungi in agricultural ecosystems (Evans & Miller 1988; Fairchild & Miller 1988; Fairchild & Miller 1990; McGonigle *et al.* 1990; O'Halloran *et al.* 1986).

The effects of disturbance on AM fungi in agricultural systems may not be universal in all habitats. Agricultural soils are dominated by AM fungi belonging to the suborder Glomineae (*i.e.* *Glomus*, *Acaulospora* and *Entrophospora*) (Helgason *et al.* 1998; Johnson & Pflieger 1992). These fungi tend to colonize plant roots through an existing external mycelium (Biermann & Lindermann 1983; Morton 1993). Such fungi would be greatly affected by any disruption of the soil, such as tilling, because it would disrupt the mycelial network, thereby compromising their ability to colonize additional roots. AM fungi that belong the Gigasporineae (*Gigaspora* and *Scutellospora*), however, may not rely on the external mycelium for root colonization. In many cases, these AM fungi have been shown to colonize exclusively from spores (Biermann & Lindermann 1983; Morton 1993). Soil disturbance may not greatly disturb their ability to colonize other roots because spores are not negatively affected by soil disturbance. Thus, existing studies that describe changes in root colonization for AM fungi in the suborder Glomineae following disturbance in agricultural systems may have little bearing on how other ecosystems respond to soil disturbance because their AM fungal community contain members of both the Glomineae and the Gigasporineae.

Further, existing studies have looked only at disturbance that occurs post-root colonization. This does not address situations where disturbance occurs prior to root colonization, such as germination immediately following tillage or in ecological situations after a large-scale disturbance.

We suggest that due to differences in AM fungal colonization strategies there is greater variation among AM fungi in how they respond to disturbance than has been previously reported. While much progress has been made in investigat-

ing the effect of disturbance at the level of the individual species of AM fungi, we believe that there may be trends which are consistent at higher taxa levels. Such general rules, if they exist, would make it easier to predict the effect of disturbance in very different ecosystems, depending on the identity of the AM fungi present. We suggest that based on basic ecological differences among AM fungi at the subfamily level, AM fungi will respond differently to disturbance prior to root colonization in the following ways:

Prediction one: AM fungi belonging to the suborder Glomineae will be more negatively affected by soil disturbance than AM fungi belonging to the suborder Gigasporineae.

Because soil disturbance breaks up the soil and fractionates extra-radicle mycelia, AM fungi that colonize roots using extra-radicle 'runner hyphae' (Glomineae) may be more severely affected than AM fungi that colonize roots from spores (Gigasporineae). Following disturbance, it is expected that some hyphal fragments will lose viability once severed due to cytoplasmic leakage whereas spores should not be affected. Thus, AM fungi that depend upon their external mycelium for colonization would be more compromised by disturbance compared to AM fungi that easily colonize via spores. That is, following disturbance, spore colonizers re-establish as they would in the absence of disturbance whereas mycelium-colonizers must first rebuild a mycelium in order to be highly infective.

Prediction two: Host plants associated with AM fungi belonging to the suborder Glomineae will be more negatively affected by soil disturbance than host plants associated with AM fungi belonging to the suborder Gigasporineae.

We also propose that there will be variation in host plant response to disturbance, depending on their AM fungal associates. Plants associated with AM fungi in the suborder Glomineae may be more negatively affected, in terms of nutrient status/and or biomass, than plants associated with AM fungi in the suborder Gigasporineae.

The objective of this study is to test these two predictions by measuring and comparing response to soil disturbance for two host plant-AM fungal systems: one with fungal isolates from the suborder Glomineae and the other with isolates from the suborder Gigasporineae.

Materials and methods

This study was part of a larger study investigating the life history strategies of AM fungi (Hart & Reader 2002; Hart & Reader *in press*).

AM fungal isolate

We acquired 16 isolates from the suborder Glomineae and 5 isolates from the suborder Gigasporineae listed below from a collection at the University of Guelph and Premier Tech. Inc. (see Hart & Reader *in press*). The following isolates were chosen to include representatives of three general within the Glomineae and two genera within the Gigasporineae and to provide replication within the suborder.

Suborder Glomineae

<i>Acaulospora morrowae</i>	LTMRs*
Spain and Schenck	
<i>Acaulospora spinosa</i> 1	LTMRs
Walker and Trappe	
<i>Acaulospora spinosa</i> 2	LTMRs
<i>Entrophospora columbiana</i>	LTMRs
Spain and Schenck	
<i>Glomus aggregatum</i>	LTMRs
Schenk & Smith emend. Koske	
<i>Glomus claroideum</i>	LTMRs
Schenk & Smith	
<i>Glomus constrictum</i> Trappe	LTMRs
<i>Glomus etunicatum</i>	LTMRs
Becker & Gerdemann	
<i>Glomus geosporum</i>	LTMRs
(Nicol. & Gerd.) Walker	
<i>Glomus intraradices</i>	Premier Tech. Inc.**
Quebec1 (Q1) Schenck & Smith	
<i>Glomus intraradices</i> Quebec 2 (Q2)	Premier Tech. Inc.
<i>Glomus intraradices</i> Kansas (K)	Premier Tech. Inc.
<i>Glomus intraradices</i> Isreal (I)	Premier Tech. Inc.
<i>Glomus intraradices</i> France (F)	Premier Tech. Inc.
<i>Glomus intraradices</i> (J)	Premier Tech. Inc.
<i>Glomus mosseae</i> (Nicol. & Gerd.)	LTMRs
Gerdemann & Trappe	

Suborder Gigasporineae

<i>Gigaspora gigantea</i> (Nicol. & Gerd.)	LTMRs
Gerdemann & Trappe	
<i>Gigaspora margarita</i>	LTMRs
(Becker & Hall)	
<i>Scutellospora calospora</i>	LTMRs
(Nicol. & Gerd.) Walker & Sanders	
<i>Scutellospora heterogama</i>	LTMRs
(Nicol. & Gerd.) Walker & Sanders	
<i>Scutellospora pellucida</i> (Nicol. & Schenck) Walker & Sanders	LTMRs

* LTMRs (Long-term mycorrhizal research station, Department of Botany, University of Guelph, Guelph, Ontario Canada)

** Premier Tech. Inc. Riviere-du-Loup, Quebec Canada

Inoculum

For both suborders, we used whole inoculum (root fragments, hyphal fragments and spores) to prevent biasing the experiment in favour of one suborder. The amount of inoculum added was equalized among isolates in terms of fungal biomass (ergosterol). Samples of whole inoculum from each isolate were homogenized, and the ergosterol was extracted with hexane by HPLC following the method of Grant & West (1986). The standard used was commercial ergosterol (5,7,22-ergostatrien-3 β -ol). Once we determined the concentration of ergosterol in whole inoculum, each isolate was diluted down to a common ergosterol density. We did not equalize in terms of inoculum potential (amount of colonization per unit propagule) since this might reflect an important aspect of colonization strategies for AM fungi.

In all cases isolates were cultured prior to the experiment by growing the inoculum with a surrogate host. We chose leek (*Allium porrum* L.) as the surrogate host as it is commonly used in trap cultures and is known to host a wide variety of AM fungi. Surrogates were grown in containers (4 cm x 20.5 cm) (Stuewe & Sons Inc. Corvallis Oregon USA) which were two-third filled with a sterile, low P potting soil and silica sand mixture in a 1:1 ratio. Throughout the experiment, soil was sterilized by heating it to 120 °C for 30 minutes, left to cool overnight, then 120 °C again for 30 minutes at which point it was left for 1 week prior to planting. At this point, a layer of inoculum was added to each container and each pot was filled with additional soil-sand mixture. We then added three *Allium* seedlings, which were grown for 30 days, at which point they were harvested by entire removal of the shoot by hand. The 'cultured' soil in the containers was then subjected to the experimental treatments.

Experimental treatments

Three experimental treatments were applied to containers using a completely randomized factorial design with five replicates for each experimental treatment. The three experimental treatments were (1) AM fungal isolate (2) disturbance treatment and (3) host plant.

AM fungal isolate

We used 21 different AM fungal isolates as described above. Spores were examined at the end of

the experiment to verify the identity of the test isolates.

Disturbance treatment

We used two different disturbance regimes: disturbed and intact soil. For both disturbance treatments, soil was prepared with inoculum and a surrogate host as described above. At thirty days the surrogate was harvested. For *intact* treatments experimental seedlings were planted directly into the pre-cultured soil. For *disturbed* treatments, soil was passed through a 250 μ m sieve and then replanted with an experimental seedling.

In this study, soil disturbance involves the destruction of the root. Any subsequent colonization by AM fungi must occur on a new, uninfected root. This is a common type of disturbance in agricultural systems and natural settings. For our purposes, it meant that AM fungi could not propagate from pre-existing infections through hyphal runners, thus isolating the differences in germination strategies among the AM fungal suborders.

Host Plant

We used four host plants representing two growth habits: grasses and forbs. We chose two species from each growth habit for the purposes of replication. These were: English plantain (*Plantago lanceolata* L.), common plantain (*Plantago major* L.), Kentucky-blue grass (*Poa pratensis* L.) and annual blue grass (*Poa annua* L.). These species were chosen as highly mycotrophic plants that were common to old fields and meadows (which was the origin of most of the AM fungal isolates). Three seedlings of each species (approximately 1 cm radicle) were added to each container, at which point 50 ml of soil filtrate (mesh size 35 μ m) from each isolate was added to each container to control for differences in other soil organisms among containers. Plants were randomized on 14 greenhouse benches (1.5 m by 8 m) at Premier Tech., Riviere-du-Loup, Quebec, Canada. Each container was subjected to 14 hours of light (12.2 Watts m⁻² over a 24 hour period) for 12 weeks between July and October 2000. Containers were watered and fertilized as needed with a low P fertilizer.

Control for background fungi

It is possible that some non-Glomalean fungi may be counted in ergosterol measurements of root and fungal soil biomass. To estimate the amount of fungi other than AM fungi in our treatments, we ran concurrent control containers, similar in every

way to treatment containers except they were lacking AM fungi.

Dependent variables

The response of AM fungi to soil disturbance was measured in terms of (1) intra-radicle colonization measured by % intra-radicle colonization and intra-radicle fungal biomass, (2) extra-radicle colonization measured by soil hyphal length and soil fungal biomass and (3) spore density. We looked at two measures each of intra- and extra-radicle colonization because we wanted concurrent measurements of fungal biomass (by which our treatments were equalized) and hyphal extent.

Extent of intra-radicle colonization

Extent of intra-radicle colonization was determined at the final harvest (week 12) using % colonization and root fungal biomass. Percent colonization was assessed using the following method. After harvesting, the entire root system was cleaned by first shaking off excess soil and then washing the roots with water. Finally, the roots were sonicated for 15 seconds to remove any residual matter, including hyphae. The clean root system was then cut into 2 cm fragments from which eight were randomly selected to determine % colonization. These root fragments were stained with Chlorazol Black (Brundrett 1991), and then quantifying fungal presence using a gridline intersect method for 100 intersections on two slides. (McGonigle *et al.* 1990). Percent whole colonization was determined as any portion of the AM fungal mycelium (hyphae, spores of vesicles) in the root. Fungal root biomass was assessed by measuring the amount of ergosterol. Eight 2 cm fragments of clean roots were randomly selected for root fungal biomass analysis (see above section). These were then homogenized before extraction following the method cited above.

Extent of extra-radicle colonization

Extent of extra-radicle colonization was determined at final harvest (week 12) using soil hyphal length and soil fungal biomass. Soil hyphal length was estimated by measuring lengths of hyphae by a modified grid line intersect method as per Klironomos *et al.* 1993. Two 5 g portions of soil were removed from each container and suspended in 250 ml of water. Sodium hexametaphosphate (3.6% w/v) was added and left for approximately to break up soil aggregates. The soil suspension was then agitated in a blender at high speed for two minutes

and then stirred with an electronic stir bar. One 6 ml aliquot per sample was removed halfway between the beaker edge and the vortex and to this was added 250 ml distilled water and 30 ml sodium hexametaphosphate solution. This was stirred again to resuspend hyphae then 10 ml aliquots were taken and transferred to 50 ml centrifuge tubes where they were centrifuged at 1000 x g until no more hyphal fragments were extracted (five times). The pellets were resuspended in glycerol and again centrifuged (75 x g for 30 seconds). The supernatant was filtered onto a 20- μ m polyester filter which was then stained with Chlorazol Black and then decanted over a 1.2 μ m nitrocellulose filter paper. These were then mounted on glass slides, dried and made transparent by mounting in immersion oil. Using the grid line intersection (70 intersections/slide) two slides were examined for each container. Hyphal length per gram dry soil was calculated as in Newman (1966).

The biomass of the extra-radicle mycelium was determined by the same extraction methods given for root biomass using instead 20 ml of root-free soil.

Spore density

To assess differences in sporulation following disturbance we calculated spore density at the final harvest (week 12). Spore density was calculated by sucrose fractionation (Klironomos *et al.* 1993). 30 ml of soil was removed from each container and agitated in a blender with 250 ml of water for one minute. Each suspension was wet sieved through four sieves (1 mm, 500 μ m, 250 μ m and 45 μ m) and then resuspended in 50 ml of water. It was then divided between two 50 ml centrifuge tubes and floated on top of a 60% sucrose solution and spun at 688 x g for 20 minutes. Spores were then filtered onto gridded (3 x 3 mm) 1.2 μ m filters. Spores were quantified by counting all visible spores in 10 randomly chosen squares on the grid.

Response of plant hosts

The response of plant hosts to soil disturbance was measured in terms of: (1) biomass and (2) % foliar phosphorus. We chose to look at two measures of plant response since association with AM fungi might not realize benefits in biomass but may confer improved nutrient status. Host plants were harvested after 12 weeks, separated into shoots and roots, dried at 60 °C for 48 hours and

weighed. Foliar phosphorus concentration (% of dry weight) was calculated by measuring bicarbonate-extractable phosphorus as per Olsen & Sommers (1982). Shoots were dried as described above and 0.4 g of leaves were randomly chosen for phosphorus analysis. Where there was not sufficient dry leaf mass to make up 0.4 g, all leaves were used.

Data Analysis

To quantify the response of AM fungi and host plants to soil disturbance, the following metric was calculated for each of the five dependent variables:

$$\text{Change} = (\text{Disturbed} - \text{Intact}) / (\text{Intact})$$

where, Disturbed = the value of each replicate in a disturbed treatment; Intact = the average value of all five replicates within a corresponding intact treatment. The average value was used because our disturbed and undisturbed experimental units were not paired in this experimental design.

Statistical analysis

We used SPSS 7.0 in all analyses (SPSS 1995). For all analyses data were taken as frequency of occurrence. For graphical purposes, however, these frequencies were converted to percentages, due to large differences in sample size among the suborders.

Prediction one: AM fungi belonging to the suborder Glomineae will be more negatively affected by disturbance than AM fungi belonging to the suborder Gigasporineae.

To test this prediction, we compared the frequency distribution of 'change' values for the two AM fungal suborders using a G-test (Sokal & Rohlf 1995). A separate G-test was performed for % root colonization, root fungal biomass, soil hyphal length, soil fungal biomass and sporulation density.

Prediction two: Host plants associated with AM fungi belonging to the suborder Glomineae will be more negatively affected by disturbance than host plants associated with AM fungi belonging to the suborder Gigasporineae.

To test this prediction, we compared the frequency distribution of 'change' values for host plants grown with Glomineae versus Gigasporineae isolates. A separate G-test was performed for plant biomass and % foliar phosphorus.

Relationship between responses of AM fungi and host plants to soil disturbance

A separate analysis was performed for all possible combinations of the five measures of AM fungal colonization (% root colonization, soil hyphal length, root fungal biomass, soil fungal biomass and spore density) and two measures of host plant response (biomass, % foliar phosphorus). To determine the strength of the relationship between response of AM fungi and host plants to soil disturbance, Pearson's product-moment correlation coefficient was calculated using 'change' values (calculated as described above).

We considered both suborders together for this analysis because our objective was to test for a relationship between the responses of AM fungi and plants hosts to soil disturbance regardless of AM fungal identity.

Host effect

Because four different plant hosts were used in this study, we tested whether host identity was a significant source of variation in our results. We examined the effect of host identity using a two-way analysis of variance (ANOVA) identity and fungal isolate as the factors. A separate ANOVA was run for each of the dependent variables.

Results

Effect of soil disturbance on AM fungal colonization

The following description of results is based on pooled data for all four host plants because the host*AM fungal suborder interaction term in ANOVA was not statistically significant for any of the dependent variables (% AM fungal colonization ($F_{48, 419} = 1.11$, $P=0.30$), soil ergosterol ($F_{48, 419} = 0.56$, $P=0.99$), root ergosterol ($F_{48, 419} = 0.56$, $P=0.99$), soil hyphal length ($F_{48, 419} = 1.05$, $P=0.39$), and spore density ($F_{48, 419} = 0.62$, $P=0.98$)).

% Root colonization and fungal root biomass

AM fungi in the Glomineae were significantly more negatively affected by soil disturbance than AM fungi in the Gigasporineae with regard to their ability to colonize new roots ($G = 22.22$, $P < 0.001$) (Fig. 1a). This was also more evident in their ability to accumulate biomass in these new roots (Fig. 1b) ($G = 11.6$, $P < 0.01$).

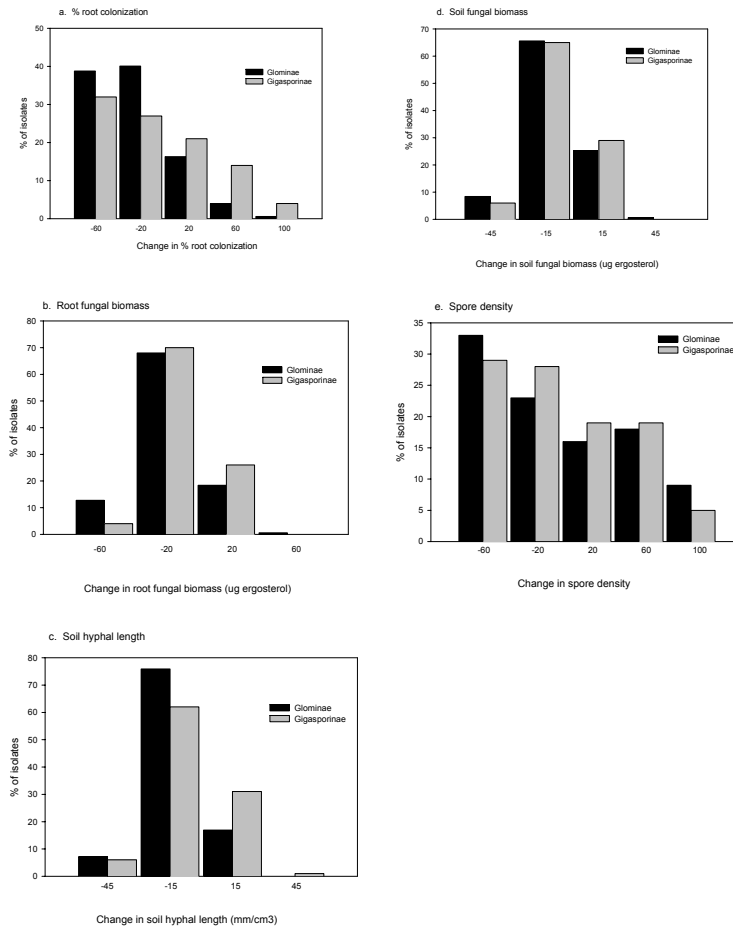


Fig. 1. Frequency distribution of change in AM fungal performance due to soil disturbance for 16 Glomineae isolates and 5 Gigasporineae isolates. a. % root colonization, b. root fungal biomass, c. soil hyphal length, d. soil fungal biomass, e. spore density.

Fig. 1a shows large variation among individual isolates in their response to soil disturbance with values ranging from negative to positive. On the negative side, 40% of Glomineae and 27% of Gigasporineae isolates experienced a decrease of root colonization following soil disturbance of between 0 and 400% compared to intact soil values. Some Glomineae (38%) and Gigasporineae (32%) were severely affected by soil disturbance with between 400% and 80% lower root colonization. On the positive side, 21% (Glomineae) and 41% (Gigasporineae) of isolates had invigorated % colonization of up to 160 % greater than in undisturbed soil. Average values for individual isolates are given in Table 1.

Similar trends were evident in fungal root biomass (Fig. 1b). Most isolates were negatively affected by disturbance, with 68% (Glomineae) and 70% (Gigasporineae) of isolates experiencing a decrease in root ergosterol after disturbance between 0 and 400 µg ergosterol g⁻¹ dry root weight. On the positive side, invigorated internal colonization following disturbance was also detected in root fungal biomass with isolates showing increases in root fungal biomass of up to 80 µg ergosterol (19% of Glomineae isolates) and 40 µg ergosterol (29% of Gigasporineae isolates). See individual isolate responses in Table 1.

Table 1. Change in AM fungal per cent root colonization, root fungal biomass, soil hyphal length, soil fungal biomass and spore density from non-mycorrhizal controls following soil disturbance for individual AM fungal isolates. Values given have been pooled for all host plants. Values given are mean values for N=5 with 1 standard error of the mean given in brackets.

	Change in AM fungal colonization (% of control)				
	% Root colonization	Root fungal biomass	Soil hyphal length	Soil fungal biomass	Spore density
<i>Glomus intraradices Q1</i>	-9 (2)	-8(2)	-7(2)	-12(2)	-3(11)
<i>Scutellospora pellucida</i>	39(7)	-1(2)	1(2)	1(2)	-12(13)
<i>Glomus constrictum</i>	-13(4)	-8(3)	-7(2)	-4(2)	-11(15)
<i>Glomus mosseae</i>	-53(3)	-38(2)	-23(1)	-23(3)	17(14)
<i>Glomus aggregatum</i>	-50(4)	-32(2)	-17(2)	-11(3)	-11(16)
<i>Glomus claroideum</i>	-40(4)	-21(3)	-13(2)	-7(2)	-11(9)
<i>Gigaspora gigantea</i>	-6(17)	-9(2)	-4(2)	-4(2)	-18(11)
<i>Glomus intraradices K</i>	-8(4)	-6(2)	-9(2)	-5(2)	4(10)
<i>Acaulospora spinosa 1</i>	-51(6)	-21(3)	-14(2)	-13(3)	-16(10)
<i>Scutellospora calospora</i>	15(6)	-4(2)	11(13)	-3(1)	7(12)
<i>Glomus geosporum</i>	13(10)	6(5)	-4(3)	-3(4)	-19(14)
<i>Scutellospora heterogama</i>	-22(4)	-13(2)	-12(2)	-9(2)	-18(12)
<i>Acaulospora spinosa 2</i>	-41(6)	-20(3)	-15(2)	-14(3)	-16(13)
<i>Gigaspora margarita</i>	-1(0)	-31(1)	-27(1)	-2(1)	-2(13)
<i>Glomus intraradices I</i>	-68(5)	-43(3)	-22(2)	-19(3)	-15(13)
<i>Entrophospora columbiana</i>	-16(7)	-11(3)	-10(2)	-7(3)	-2(13)
<i>Glomus etunicatum</i>	-5(5)	-8(4)	-6(2)	-9(2)	31(20)
<i>Glomus intraradices F</i>	-36(4)	-24(3)	-14(1)	-13(2)	-14(11)
<i>Glomus intraradices Q2</i>	-46(7)	-28(4)	-12(2)	-14(2)	-12(12)
<i>Glomus intraradices J</i>	-34(4)	-24(3)	-15(2)	-15(3)	2(14)
<i>Acaulospora morrowaie</i>	10(16)	-5(4)	-6(3)	2(3)	2(10)

Hyphal length and fungal biomass in the soil

Changes in soil hyphal length in response to soil disturbance differed significantly between the two suborders of AM fungi ($G=11.96$, $P<0.01$). Again, more AM fungi in the Glomineae were negatively affected by disturbance compared to AM fungi in the Gigasporineae (Fig. 1c). A similar trend was evident for fungal soil biomass but the difference between suborders was not statistically significant ($G=1.94$, $P<0.9$) (Fig. 1d).

For both hyphal length and biomass, we found large variation among AM fungi isolates that ranged from negative to positive values. On the negative side, soil hyphal length decreased between 0 and 30 mm cm⁻³ soil for most isolates in both Glomineae (76%) and Gigasporineae (62%). This was also true for soil fungal biomass

(Glomineae 66% and Gigasporineae 65%). On the positive side, some isolates had increased soil colonization following soil disturbance with 17% of Glomineae and 32% of Gigasporineae isolates increasing in soil hyphal length. This was true also for soil fungal biomass, where 25% (Glomineae) and 29% (Gigasporineae) isolates had increased root ergosterol following disturbance. Individual isolate values for soil hyphal length and soil fungal biomass are given in Table 1.

Spore density

The change in spore density due to soil disturbance did not differ significantly between AM fungi in the Glomineae versus Gigasporineae ($G=29.8$, $P>0.99$) (Fig. 1e).

Among isolates we found large variation in sporulation in response to disturbance, again ranging from positive to negative. On the negative

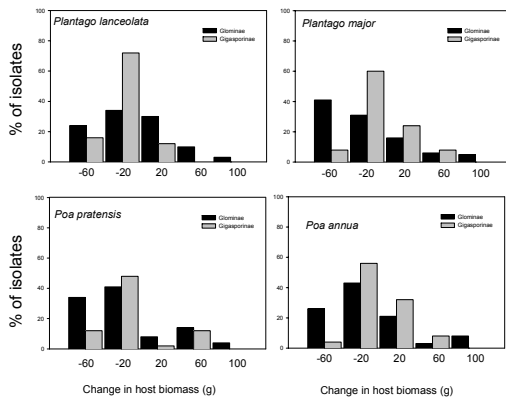


Fig. 2. Frequency distribution of change in the biomass of four host plants when grown individually with Glomineae isolates versus Gigasporineae isolates. a. *Plantago lanceolata*, b. *Plantago major*, c. *Poa pratensis*, d. *Poa annua*.

side, 55% of the Glomineae and 57% of the Gigasporineae isolates had a large reduction in spore density following soil disturbance of between 0 and 80 spores/30mL soil. On the positive side, 45% of the Glomineae and 43% of the Gigasporineae isolates had a greater spore density following soil disturbance. The response of individual isolates are listed in Table 1.

Effect of disturbance on plant host response

The biomass of host plants differed significantly ($F_{3, 419}=3.2$, $P=0.02$) so responses of the four hosts are shown separately in Fig. 2.

Host plant biomass

The response of plant hosts to soil disturbance differed significantly with respect to the two AM fungal suborders (Fig. 2). For each of the four host plants, the biomass of plants growth with AM fungi from the Gigasporineae was less negatively affected than hosts grown with AM fungi in the Glomineae (*Plantago lanceolata* $G=16.8$, $P<0.001$, *Plantago major* $G=16.6$, $P<0.001$, *Poa pratensis* $G=27.6$, $P<0.001$, *Poa annua* $G=13.6$, $P<0.001$).

For each host, their response to disturbance ranged from negative to positive (Fig. 2). For all hosts combined, 31% of plants grown with Glomineae isolates and 10% of plants grown with Gigasporineae isolates had reduced biomass in excess of 40 g compared to non-disturbed plants in response to disturbance. On the positive side 32% of plants grown with Glomineae isolates and 28%

of plants grown with Gigasporineae isolates had an increase in biomass of compared to undisturbed treatments. The response of host biomass to individual isolates is given in Table 2.

Host plant % foliar phosphorus

The concentration of foliar phosphorus did not differ significantly among the four host plants ($F_{3,419}=0.77$, $P=0.51$) so data for hosts has been pooled in Fig. 3. For % foliar phosphorus, host plant response to soil disturbance did not differ significantly with respect to the two AM fungal suborders ($G = 4.8$, $0.05<p<0.1$) (Fig. 3). Each host plant also experienced a wide range of change in % foliar phosphorus following soil disturbance. On the negative side, 40% of plants grown with Glomineae isolates and 46% of plants grown with Gigasporineae isolates had reduced foliar phosphorus concentration of between 15 % and 60% compared to plants grown in undisturbed soil. On the positive side 44% of hosts associates with Glomineae isolates and 43% of hosts associates with Gigasporineae isolates had elevated foliar phosphorus concentration compared to plants grown in undisturbed soil. The increase in % phosphorus ranged from 75% to 120% for 3% of the plants grown with Glomineae isolates and 1% of plants grown with Gigasporineae isolates. See Table 2 for the effect of individual isolates on host phosphorus.

Relationship between responses of AM fungi and host plants to soil disturbance

Responses of AM fungi and host biomass to soil disturbance were significantly correlated in some cases, but not in others, for each of the four plant hosts (Table 3). *Poa pratensis* biomass was positively correlated with change in % root colonization, change in soil hyphal length and change in root fungal biomass. *Poa annua* biomass was also positively related to change in AM fungal colonization (percent root colonization, root fungal biomass and soil fungal biomass) (Table 3a). For *Plantago lanceolata*, however, host biomass was negatively correlated with change in root fungal biomass. *Plantago major* was not correlated with any measure of AM fungal colonization.

For all hosts combined, host foliar phosphorus concentration response to soil disturbance was correlated with AM fungal responses for three of the five measures of AM fungal response (root fungal biomass, soil fungal biomass and spore density) (Table 3b).

Table 2. Change in host response from non-mycorrhizal controls following soil disturbance for individual AM fungal isolates. Values for host biomass are given for each host independently, while those for host phosphorus have been pooled. Values given are mean values for N=5 with 1 standard error of the mean given in brackets.

	Change in biomass (% of control)				Change in foliar phosphorus (% of control)
	<i>Plantago lanceolata</i>	<i>Plantago major</i>	<i>Poa pratensis</i>	<i>Poa annua</i>	All hosts
<i>Glomus aggregatum</i>	-3(4)	-1(8)	-19(5)	-6(5)	9(8)
<i>Glomus claroideum</i>	23(5)	-25(3)	53(10)	-23(4)	4(7)
<i>Glomus constrictum</i>	-56(3)	29(6)	-49(3)	33(4)	2(8)
<i>Glomus etunicatum</i>	1(3)	-33(7)	-31(6)	-22(7)	-4(6)
<i>Glomus geosporum</i>	-22(5)	-47(4)	1(4)	24(5)	5(9)
<i>Glomus intraradices Q1</i>	-15(7)	41(13)	-32(6)	-25(7)	-9(6)
<i>Glomus intraradices Q2</i>	54(14)	-93(0)	62(9)	-82(2)	5(6)
<i>Glomus intraradices I</i>	53(4)	-27(5)	-20(5)	-15(3)	-7(5)
<i>Glomus intraradices F</i>	-68(2)	206(60)	-51(4)	-66(2)	-1(7)
<i>Glomus intraradices J</i>	37(6)	-76(3)	-68(1)	-67(4)	-12(6)
<i>Glomus intraradices K</i>	2(4)	-43(8)	64(6)	-26(7)	10(8)
<i>Glomus mosseae</i>	-28(6)	27(4)	-36(2)	-24(3)	-1(8)
<i>Acaulospora spinosa 1</i>	18(7)	-52(3)	-6(5)	-51(4)	-3(7)
<i>Acaulospora spinosa 2</i>	-12(6)	-12(2)	-34(7)	7(3)	3(6)
<i>Acaulospora morrowaie</i>	-21(7)	-47(8)	-33(10)	31.3(20)	-11(4)
<i>Entrophospora columbiana</i>	-75(2)	-73(1)	-7(1)	200(21)	5(8)
<i>Gigaspora margarita</i>	-36(4)	34(4)	-41(3)	8(4)	-11(6)
<i>Gigaspora gigantea</i>	-19(6)	-8(4)	16(6)	37(8)	-6(6)
<i>Scutellospora pellucida</i>	-22(5)	-30(7)	45(7)	-16(6)	-7(5)
<i>Scutellospora heterospora</i>	-8(7)	-18(8)	-20(7)	-16(2)	-7(5)
<i>Scutellospora calospora</i>	-31(8)	-19(6)	7(6)	-20(8)	-5(6)

Background fungi

Our control pots contained a small amount of background fungi. Root ergosterol was measured to range from 0.07 – 0.41 $\mu\text{g g}^{-1}$ dry root and soil ergosterol ranged from 0.15 – 0.37 $\mu\text{g g}^{-1}$ dry soil. However, we are confident that these values did not represent any AM fungi as we observed no intra-radicle colonization by AM fungal structures (arbuscules, vesicles, coils).

Discussion

Effect of soil disturbance on AM fungal colonization

Our results confirm that there is considerable variation among AM fungi in their response to soil disturbance. Previous studies that focused on the suborder Glomineae reported only a fraction of

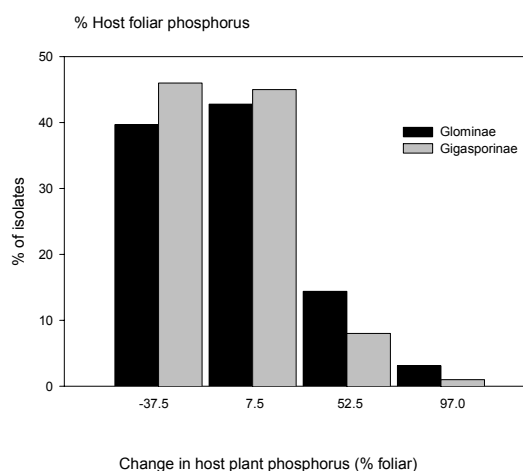


Fig. 3. Frequency distribution of change in host plant foliar phosphorus when grown with Glomineae isolates versus Gigasporineae isolates. Data for all four hosts has been pooled.

Table 3. Pearson's product-moment correlation coefficients for bivariate correlations between the change in host plant response (biomass and phosphorus concentration) and change in AM fungal colonization (% root colonized, root fungal biomass, soil hyphal length and soil fungal biomass) following soil disturbance. *signifies significance at $P < 0.05$, **signifies significance at $P < 0.01$, and ***signifies significance at $P < 0.001$.

AM fungal variable	<i>Poa pratensis</i>	<i>Poa annua</i>	<i>Plantago lanceolata</i>	<i>Plantago major</i>
a. Host biomass				
% root colonization	0.21*	0.23*	-0.16	-0.07
Soil hyphal length	0.24**	0.01	-0.06	-0.10
Root fungal biomass	0.18	0.30**	-0.33***	-0.16
Soil fungal biomass	0.12	0.24*	0.04	-0.04
Spore density	0.01	-0.03	0.02	-0.10
b. Host phosphorus concentration				
AM fungal variable	All host plants			
% root colonization	0.03			
Soil hyphal length	0.03			
Root fungal biomass	0.49***			
Soil fungal biomass	0.58***			
Spore density	0.57***			

that variation. The suborder Gigasporineae is less sensitive to soil disturbance, as we predicted. The basis for this difference between suborders is likely due to differences in their colonization strategies. AM fungi in the Gigasporineae colonize primarily from spores whereas AM fungi in the Glomineae can colonize from hyphae (Biermann & Lindermann 1983; Klironomos & Hart unpublished; Morton 1993; Tommerup & Abbot 1981). Hyphae are more sensitive than spores to soil disturbance and thus subsequent colonization of additional roots is more affected. These results are particularly convincing because roots were harvested quite late (at week 12). Even though such a late harvest increased the chances that mycorrhizas would be formed by secondary colonization rather than by soil propagules, we observed a significant difference between the two AM fungal suborders in the amount of root colonization.

Within each suborder, there was also considerable variation in their response to soil disturbance. The change in colonization due to soil disturbance ranged from -100% of undisturbed values to over 100% of undisturbed values for both Glomineae and Gigasporineae. Part of this variation was due to differences among AM fungal isolates in their response to soil disturbance (Table 1). The biological basis for his inter-isolate variation requires further study.

It is important to point out that we examined only one type of soil disturbance; namely, the physical rearrangement of soil causing the complete destruction of plant roots. In situations where soil disturbance is less severe such that some roots remain intact, AM fungi in the suborder Glomineae may not be disadvantaged compared to AM fungi in the suborder Gigasporineae. In such situations, isolates in the Glomineae may be able to colonize new roots more quickly than isolates in the Gigasporineae since Glomineae isolates could potentially send out runner hyphae more quickly than the Gigasporineae which rely on spores to colonize new roots. To this end, some Glomineae have been shown to be the most tolerant to repeated soil disturbance in an agricultural setting. Helgason *et al.* (1998) showed that arable fields in N. England were dominated by a Glomineae genotype that was poorly represented in surrounding woodlands. If disturbance occurred after AM fungi were established in a root, then AM fungi which colonize chiefly by 'runner' hyphae (i.e., the Glomineae) may not be disadvantaged by soil disturbance compared to the results of our study. In contrast, the Gigasporineae, which colonize roots only through spores would be equally affected by severe and less severe soil disturbance. In either case, these fungi would need to re-establish new infections from spores.

Effect of soil disturbance on host plants

Our results support the prediction that host plants associated with disturbance-resilient AM fungi are also more resilient to soil disturbance in terms of biomass. Plant biomass was less negatively affected when hosts were grown with isolates in the Gigasporineae which were less negatively affected by disturbance than the Glomineae isolates. This finding implies that plants in ecosystems lacking AM fungi in the Gigasporineae will be more vulnerable to disturbance. It also sug-

suggests that crops in agro-ecosystems might be more resistant to periodic soil disturbance if there is sufficient representation by Gigasporineae in the AM fungal community. Again, there was some intra-family isolate variation on host response to soil disturbance (Table 3). Whether this is due to intra-family variation in AM fungal response to disturbance, or to other factors requires further study.

In term of phosphorus concentration we did not find that plants associated with isolates in the Gigasporineae were more resilient to soil disturbance than plants associated with AM fungi in the Glomineae. In our study, many of the plants in both the Glomineae and the Gigasporineae had improved levels of foliar phosphorus following disturbance, despite a reduction in AM fungal colonization. Thus, our study contradicts previous studies which show that soil disturbance results in a loss of host phosphorus (Evans & Miller 1988; Evans & Miller 1990; Fairchild & Miller 1988; O'Halloran & Miller 1986). When one considers the concomitant loss of biomass for plants with increased phosphorus concentration, it is possible that these plants have not gained phosphorus; rather their foliar phosphorus is simply more concentrated in a smaller plant. Such an increase in phosphorus without increased biomass would be common when factors other than phosphorus are limiting photosynthesis and biomass accumulation. Some plants associated with Glomineae AM fungi in our study experienced both an increase in foliar phosphorus and an increase in total biomass while also experiencing a decrease in AM fungal colonization. Similar phenomena have been reported by McGonigle *et al.* (1990), Vivekanandan & Fixen (1991) & McGonigle & Miller (1993).

Clearly AM fungal response to disturbance is not the only factor influencing host response. Certainly, the response of host plants to AM fungal colonization is more complicated than merely the degree of colonization. Despite this fact, however, we were able to detect a difference in host biomass due to soil disturbance.

Relationship between AM fungi and host plant responses to soil disturbance

When we compared AM fungi and host plant responses to disturbance, host biomass was positively correlated with AM fungal colonization for the two *Poa* species. Presumably, AM fungi that

continue to maintain their original levels of internal and external colonization following soil disturbance would continue to supply their hosts with nutrients for photosynthesis. However, for *Plantago lanceolata*, biomass was negatively correlated with AM fungal colonization and for *Plantago major* there was no relationship between biomass and any AM fungal response following disturbance. This was unexpected since all of the hosts are highly mycorrhizal and as such should benefit from association with AM fungi. Perhaps the AM fungal isolates chosen for our study were largely incompatible with *P. lanceolata* and served more as a carbon drain than a mutualist. Such variation in plant response to AM fungal inoculation is becoming more prevalent in the literature (Francis & Read 1995; Klironomos 2000; van der Heijden *et al.* 1998).

For host phosphorus, there was a significant, positive relationship between AM fungal root fungal biomass, soil fungal biomass and spore density and host phosphorus. Presumably increased hyphal area both within and outside of roots could serve to increase exchange rates between host and AM fungi. The basis for a positive relationship between host phosphorus and spore density is less clear. High spore density may simply reflect a high phosphorus level within an AM fungus.

Conclusions

Differences in the colonization strategy of AM fungal suborders determines, in part, their response to soil disturbance. AM fungi that rely on spores for colonization (Gigasporineae) are less negatively affected by soil disturbance than AM fungi relying on living mycelia (Glomineae) for colonization because mycelium is more likely to be damaged by soil disturbance than spores. Thus, the composition of the AM fungal community in an ecosystem will be an important determinant of plant response to soil disturbance.

Unlike temperate systems, tropical agrosystems are highly dependent on healthy AM fungal populations for crop survival. This dependency is exacerbated by current land practices which involve partial or total removal of indigenous AM fungal populations (Cuenca *et al.* 1998; Janos 1996). Depending on the residual or introduced AM fungal community, this study suggests that crops may be more or less tolerant to disturbance.

This is a concern as it has been demonstrated that Gigasporineae are less likely to be re-introduced into a devastated area (Cuenca *et al.* 1998). Agricultural practices in tropical and temperate systems alike must move towards protecting and maintaining indigenous AM fungal populations which may be more resilient to periodic disturbance.

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