

Arbuscular mycorrhizal fungi associated with citrus orchards under different types of soil management, southern China

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ABSTRACT

Communities of arbuscular mycorrhizal fungi (AMF) were studied in sod culture (SC), straw mulching (NM), and herbicide treated and no-tillage (NH) citrus orchards, respectively. The highest total colonization rate (39.47%) and hyphal length density (1.15 m/g soil) were found in SC, the highest spore numbers (1024 spores/100 g soil) in NM, while the lowest ones (31.50%, 0.94 m/g soil and 719 spores/100 g soil) in NH and they varied significantly among three different types of orchards. Total 18 AMF species belonging to five families, Acaulosporaceae (four species), Claroideoglomeraceae (two species), Gigasporaceae (one species), Glomeraceae (nine species) and Pacisporaceae (two species) were identified, and *Glomus aggregatum* and *Claroideoglomus etunicatum* were the dominant species in all surveyed plots. The redundancy analysis showed that AMF community structure was influenced greatly by pH, soil management, soil organic matter (C_{ox}) and available phosphorus (P_{Olsen}). In SC orchards, species richness and Shannon-Wiener index of AMF were notably higher than in other treated orchards. So, it is reasonable to select SC as the best practice in citrus orchard in order to enhance AMF benefits.

Keywords: AMF diversity; colonization; spore density; hyphal length density; Satsuma mandarin

Arbuscular mycorrhizae (AM) are one of the most abundant underground symbioses, since AMF could colonize more than 80% of the higher plant species (Smith and Read 2008). They inhabit both plant roots and surrounding soils, where they can benefit their host plants in several ways, including better uptake of mineral nutrients (Mozafar et al. 2000), improving host plant photosynthesis and water status (Augé 2004, Wu and Xia 2006), increasing adverse tolerance (Gohre and Paszkowski 2006) and improving soil environment, fertility and quality (Wright and Upadhyaya 1998). Thus, AMF play an important role in the maintenance of agroecosystem stability and sustainable agricultural development.

Citrus is one of the important fruit crops in southern China, where majority of trees are cultivated in mountainous areas. Most varieties of citrus have short or even rare root hairs in field

systems, and are thus fairly dependent on AMF (Wu and Xia 2006). Clearly, the practice of sowing crop into no-till soil was developed to reduce soil erosion and costs of agricultural production (Tebrügge and Düring 1999) and under no-tillage conditions, mycorrhizal activity in soil was stimulated (Mozafar et al. 2000). In fact, both plant productivity and diversity were shown to enlarge with increasing diversity of AMF (van der Heijden et al. 2006). However, the description of the diversity of AMF in citrus orchards under different types of soil management (SM) practices was rare in last few decades in southern China.

Agricultural management factors such as the intensity of cultivation, the quality and quantity of herbicides applied and the plant protection strategies used may have severe impacts on AMF community structure (Douds and Millner 1999, Boddington and Dodd 2000, Oehl et al. 2003). In

Supported by the Science and Technology Exploitation Special Item, Project No. 2004EP090019 for Three Gorges Migrant, Ministry of Science and Technology of P.R. China.

the present study, a field survey was conducted for AM colonization in citrus roots, and for AMF spore number, hyphal length density and species diversity in citrus orchards under three different types of no-tillage SM practices, and to further propose a SM practice which can be widely employed based on benefits of AMF in citrus orchards, southern China.

MATERIAL AND METHODS

Field experiment. The investigation was carried out in hilly citrus orchards located in Wuhan (29°58'–31°22'N, 113°41'–115°05'E). This area has a semi-tropical monsoon climate. The experimental orchards planted with 15–17 year Satsuma mandarin trees (*Citrus unshiu* Marc. grafted on *Poncirus trifoliata* L. Raf.) were under three different types of no-tillage SM strategies, namely (1) sod culture 'SC' (planting 2 rows of an AM plant (Wang and Qiu 2006) Bahia grass (*Paspalum notatum* Flüggé) between trees, mowing to control the grass height and mulching under citrus trees); (2) straw-mulching 'NM' (no-tillage and straw mulching (5–10 cm) under trees in whole year except the picking time); and (3) no-tillage 'NH' (spraying herbicides e.g. Phenmedipham and Paraquat in spring and summer to suppress weeds). They were applied continuously for 5 years. The orchard soil was classified as yellow sandy clay soil (Acrisols in FAO Taxonomy) and biological organic fertilizers (7% N, 1.75% P, 3.32% K, and 20% organic matter) were applied after fruit picking (50%), before sprout (30%) and fruit setting (20%) in all experimental orchards to preserve the basic soil fertility.

Sample collection. Three randomly replicated experimental plots, where five uniform citrus trees with similar growing vigor were selected, were sampled in each SM type orchard. Fine citrus roots and rhizosphere soils from one tree were collected at four directions (east, west, south and north) from a soil layer depth of 0–30 cm after removing upper vegetation within the dripping line of the tree canopy in September, 2009. Roots and soils samples gathered from the same plot were separately pretreated before analysis. Roots ($\Phi \leq 1$ mm) were carefully washed with tap water to remove soil, chopped into 1 cm long pieces and fixed in FAA (formalin/acetic acid/ethanol, 13/5/200, v/v/v) solution for 24 h, then stored at 4°C. Soils were air-dried for 2 weeks and stored at 4°C.

Soil assessment. Selected soil chemical properties were analyzed in the Key Laboratory of Horticultural Plant Biology, Ministry of Education, Huazhong Agricultural University. Soil pH was determined by a potentiometric titration method, C_{ox} by humid oxidation with K_2CrO_7 , available nitrogen (AN) by alkaline hydrolysis diffusion method, P_{Olsen} by Olsen method, and available potassium (AK) was extracted with NH_4HCO_3 + DTPA (diethylenetriaminepentaacetic acid) and analyzed using an Inductively Coupled Plasma-Atomic Emission Spectrometer (Varian, USA).

Determination of AM colonization. Colonization of various AM fungal structures in roots was examined according to Koske and Gemma (1989) under a compound-light microscope (Olympus-BH-2, Tokyo, Japan). The ratio of root length with total AM colonization (RLT), arbuscules (RLA) and vesicles (RLV) was estimated using the magnified intersection method (McGonigle et al. 1990).

Analysis of spores. For each soil sample, spore, spore clusters and sporocarps obtained from 40 g well mixed soil using the wet sieving and sucrose gradient centrifugation technique, and the total number was counted under the stereoscopic microscope (Tech-XTS-30, Beijing, China). Thereafter, spores looking similar in size and color were separated from the spore mixture, counted and mounted onto slides using PVLG (polyvinyl alcohol-lactoglycerol) with and without Melzer's reagent (Morton 1988), and later identified using the compound microscope at 100–400 × magnification or more. AMF spores were identified up to species level based on morphological characteristics followed current descriptions and identification manuals (Schenck and Pérez 1990, Schüßler and Walker 2010, <http://invam.caf.wvu.edu>).

Ecological measures were considered to describe AMF community structure including spore numbers (SN), species richness (SR), relative abundance (RA), isolation frequency (IF) and Shannon-Wiener index (H') (Table 1). The dominant AMF species were determined according to relative abundance (RA > 20%) and isolation frequency (IF > 70%).

Quantification of hyphae length. Soil hyphal length density (HLD) was determined as described by Bethlenfalvay and Ames (1987) with the aid of an ocular micrometer under a compound-light microscope.

Statistical analysis. Data on AM colonization rates were transformed by $\arcsin x^{1/2}$. All data were statistically analyzed by variance (ANOVA) and means were compared by the least significant difference (LSD) at the 0.05 level with SAS 9.1 software

Table 1. Diversity measures used to describe arbuscular mycorrhizal fungi (AMF) communities

Measures	Formula
SR	the number of identified AMF species per 40 g soil sample
RA	RA = spore numbers of a species (genus)/the total number of identified spore samples × 100%
IF	IF = the number of soil samples where a species (genus) occurred/the total number of soil samples × 100%
H'	$H' = -\sum P_i \ln P_i$

P_i is the RA of each identified species per sampling site

(Cary, NC, USA). The relationship between AMF distribution pattern and selected environmental variables were analyzed by redundancy analysis (RDA) using the CANOCO 4.5 software (ter Braak and Šmilauer 2002).

RESULTS AND DISCUSSION

Soil chemical properties. The highest C_{ox} (23.62 g/kg) and pH value (5.74) were measured in SC, the highest AN (111.12 mg/kg), P_{Olsen} (54.93 mg/kg) and AK (182.10 mg/kg) in NM, while the lowest ones were measured in NH (Table 2). The ANOVA analysis indicated that these soil properties were significantly higher in SC and NM orchards than in NH, showing a general rule that SC and NM could improve the soil quality.

AM colonization. In our survey, all citrus trees surveyed were colonized by the indigenous AMF and formed typical AM structures such as intracellular hyphae, arbuscules and vesicles. However, vesicles and intraradical spores were rarely observed in most citrus roots.

The highest RLT, RLA and RLV (39.47%, 37.15%, and 0.54%) were all found in SC, while the lowest ones (31.50%, 21.20% and 0.10%) in NH. RLT and RLV in NM and NH orchards were significantly lower than in SC. RLA differed remarkably among three types of SM (Figure 1A). The results indicated that SM could heavily affect the AM formation in citrus roots. It is known that weed species like mycorrhizal weeds maintained in orchards might be favorable for AMF propagation and mycorrhizal symbiosis formation of citrus trees and *P. notatum* is an AM plant (Ishii et al. 2007). Thus, the gramineous species can increase the AMF propagules in SC. Additionally, citrus trees with higher AM colonization in SC orchards could easily obtain more water and mineral nutrients to improve their growth, implying the potential of AM fungi for promoting low chemical input agriculture (Atkinson et al. 2002).

In contrast, the herbicide such as Phenmedipham (used in NH) applied by foliar spray or soil dressing could inhibit photosynthesis (Ocampo and Barea 1985). Thereafter, less photosynthetic products distributed in citrus roots might indirectly suppress AM development.

Spore number and hyphal length density. SN varied significantly in all different treated orchards. The highest SN (1024 spores/100 g soil) was detected in NM, but the lowest SN (719 spores/100 g soil) still in NH (Figure 1B). It is known that AMF sporulation might be affected by different soil characteristics, the presence of other AMF species and the host (Li et al. 2007). Therefore, the higher SN was partly ascribed to the soil environment improvement in both SC and NM orchards, and then the different AMF community structures also had a severe impact on AMF sporulation.

HLD (0.94 m/g soil) in NH was notably lower than in NM and SC orchards where HLDs showed no significant difference, and the highest HLD (1.15 m/g soil) was detected in SC (Figure 1B). The remarkably higher SNs and HLDs in SC and NM orchards indicated that the formation of common mycelium network of AMF in citrus orchards might be significantly improved by the SC and NM. The higher mycelium of AMF had an important role in increasing the formation of soil aggregates (van der Heijden

Table 2. Chemical properties of the experimental soils in citrus orchards

Sites†	C_{ox} (g/kg)	Available nitrogen (mg/kg)	P_{Olsen} (mg/kg)	Available potassium (mg/kg)	pH
NH	13.04 ^b	64.90 ^c	14.33 ^c	115.15 ^b	4.83 ^b
NM	23.18 ^a	111.12 ^a	54.93 ^a	182.10 ^a	5.10 ^b
SC	23.62 ^a	97.85 ^b	29.36 ^b	162.20 ^a	5.74 ^a

†different types of soil management (SM), values in each column followed by different letters are significantly different ($P < 0.05$); NH – no-tillage; NM – straw mulching; SC – sod culture

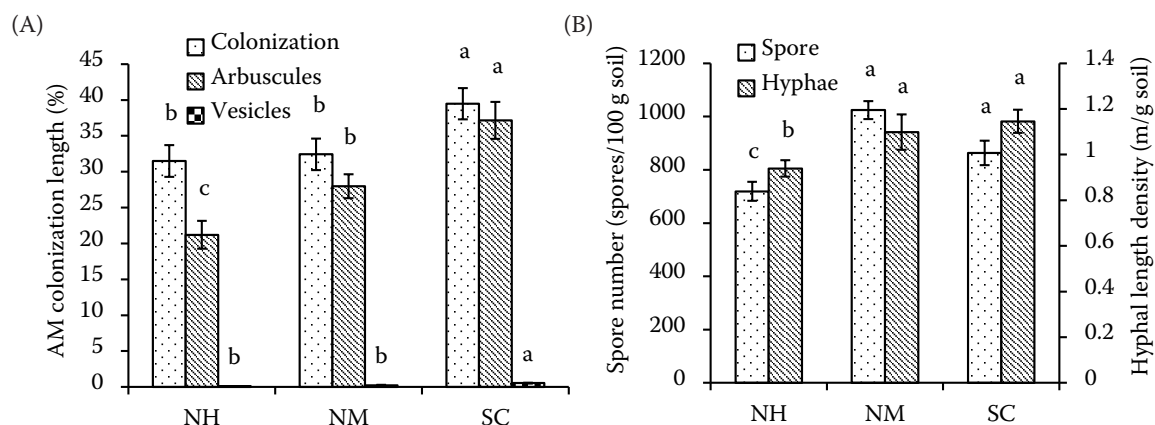


Figure 1. The different arbuscular mycorrhizae (AM) structure colonization rates (A) of citrus roots, spore number and hyphal length density (B) in three types of soil management (SM) citrus orchards. Bars with the same letter are not significantly different ($P < 0.05$); NH – no-tillage; NM – straw mulching; SC – sod culture

et al. 2006), which contributed to the maintenance of good water infiltration rates, good tilth and adequate aeration for citrus roots growth in SC and NM orchards.

RA, SR and diversity of AMF. Total 18 AMF morphological species, some of which presented in Figure 2, belonging to five fami-

lies, namely Acaulosporaceae (four species), Claroideoglomeraceae (two species), Gigasporaceae (one species), Glomeraceae (nine species) and Pacisporaceae (two species), were identified in the present investigation. Of these AMF species, 15, 12 and 11 were obtained from the SC, NM and NH orchards, respectively. Five were

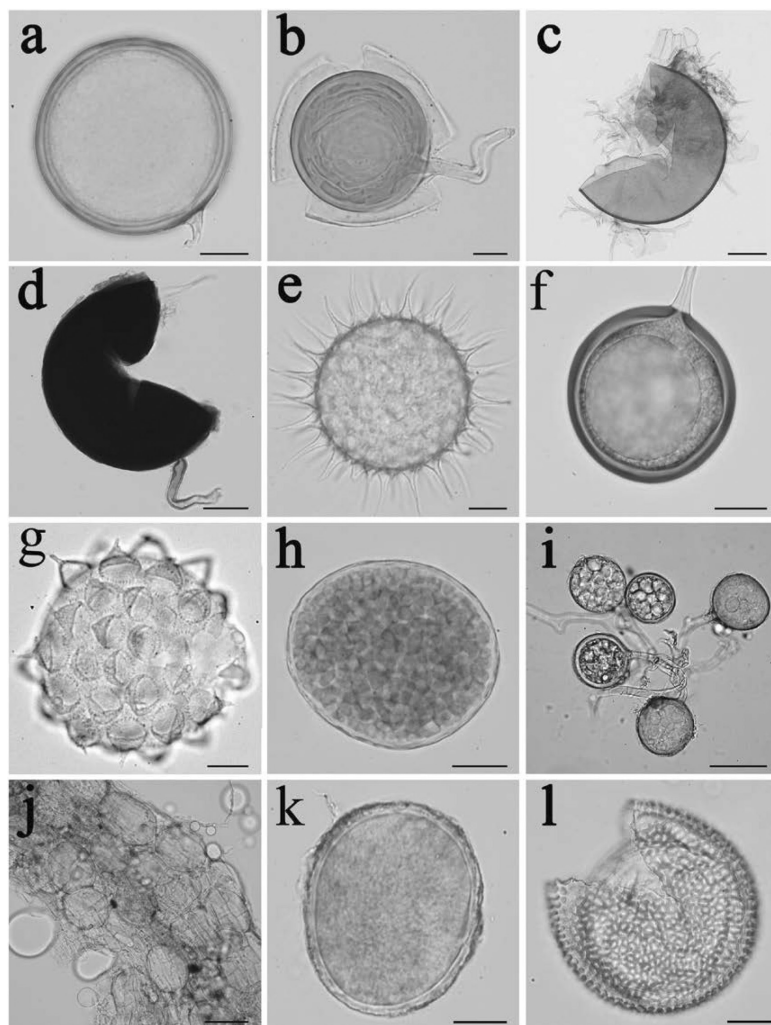


Figure 2. Some arbuscular mycorrhizal fungi (AMF) spore morphotypes detected in citrus rhizosphere. (a) *C. claroideum*, bar = 35 μ m; (b) *C. etunicatum*, bar = 35 μ m; (c) *G. tortuosum*, bar = 70 μ m; (d) *G. tenebrosus*, bar = 50 μ m; (e) *P. chimonobambusae*, bar = 30 μ m; (f) *F. geosporum*, bar = 35 μ m; (g) *Glomus* sp2, bar = 35 μ m; (h) *Glomus* sp3, bar = 40 μ m; (i) *G. aggregatum*, bar = 50 μ m; (j) *R. intraradices*, bar = 40 μ m; (k) *P. franciscana*, bar = 30 μ m; (l) *A. scrobiculata*, bar = 30 μ m

Table 3. Isolation frequency (IF) and relative abundance (RA) of the arbuscular mycorrhizal fungi (AMF) species identified from the three types of soil management (SM) citrus orchards

No.	AMF species	IF (%)			RA (%)		
		NH	NM	SC	NH	NM	SC
Acaulosporaceae							
1	<i>Acaulospora excavata</i> (Ingleby & Walker)	66.67	100	66.67	0.83	1.13	0.40
2	<i>A. scrobiculata</i> (Trappe)	0	0	100	0	0	2.60
3	<i>A. rehmsii</i> (Sieverding & Toro)	0	0	100	0	0	0.33
4	<i>A. spinosa</i> (Walker & Trappe)	33.37	0	0	0.14	0	0
Claroideoglomeraceae							
5	<i>Claroideoglomus claroideum</i> (Schenck & Smith, Walker & Schüßler)	100	100	100	15.74	18.16	10.37
6	<i>C. etunicatum</i> (Becker & Gerd., Walker & Schüßler)	100	100	100	27.64	24.31	20.69
Gigasporaceae							
7	<i>Scutellospora nigra</i> (Walker & Sanders)	0	33.33	33.33	0.78	0.78	0.13
Glomeraceae							
8	<i>Funneliformis geosporum</i> (Nicol. & Gerd., Walker & Schüßler)	33.33	100	100	0.29	2.08	2.72
9	<i>Glomus aggregatum</i> (Schenck & Smith)	100	100	100	48.44	42.84	38.02
10	<i>G. tenebrosum</i> (Thaxt., Berch)	0	0	66.67	0	0	0.53
11	<i>G. tortuosum</i> (Schenck & Smith)	0	0	100	0	0	3.80
12	<i>Glomus</i> sp1	66.67	100	0	0.78	0.78	0
13	<i>Glomus</i> sp2	33.33	66.67	66.67	0.22	0.22	0.68
14	<i>Glomus</i> sp3	66.67	33.33	0	0.21	0.21	0
15	<i>Rhizophagus diaphanus</i> (Cano & Dalpé, Walker & Schüßler)	100	100	100	2.33	1.91	1.79
16	<i>R. intraradices</i> (Schenck & Smith, Walker & Schüßler)	100	100	100	3.39	5.59	9.28
Pacisporaceae							
17	<i>Pacispora chimonobambusae</i> (Wu & Liu, Sieverd. & Oehl ex Walker, Vestberg & Schüßler)	0	100	100	0	1.72	7.93
18	<i>P. franciscana</i> (Sieverd. & Oehl)	0	0	100	0	0	0.74

NH – no-tillage; NM – straw mulching; SC – sod culture

observed only in SC: *Acaulospora scrobiculata*, *A. rehmsii*, *Glomus tenebrosum*, *G. tortuosum* and *Pacispora franciscana*, one only in NH: *A. spinosa*, and eight in all investigated orchards: *A. excavata*, *G. aggregatum*, *Claroideoglomus claroideum*, *C. etunicatum*, *Rhizophagus diaphanus*, *R. intraradices*, *Funneliformis geosporum* and *Glomus* sp2. However, *G. aggregatum* and *C. etunicatum* were the dominant species in all surveyed orchards (Table 3), and this agreed with the fact that the agricultural soils are often dominated by *Glomeraceae* species (Oehl et al. 2003).

In the present study, mean AMF SR was remarkably higher in SC than in NM and NH orchards, ranged from 6.67 in NH to 10.67 in SC (Figure 3A), and the H' (1.93) observed in SC was also notably higher than in NM and NH orchards (Figure 3B).

Furthermore, the RA of particular AMF species varied greatly among orchards (Table 3). Thus, besides the AMF species numbers, SC could change the AMF species proportion in citrus orchards. The results suggested that SC could partly recover the AMF species disappeared in long term monoculture farming. In this regard, Gosling et al. (2006) reported that long time and repeated monoculture in orchards systems could select for AMF species that provide limited benefits to the host plant. In addition, using of many agricultural practices (such as pesticides, herbicides and tillage) could make the agricultural soils AMF impoverished, especially in terms of numbers of species (Helgason et al. 1998). Hence, the most traditional agricultural practices were unfavorable to enhance the AMF benefits in citrus orchards.

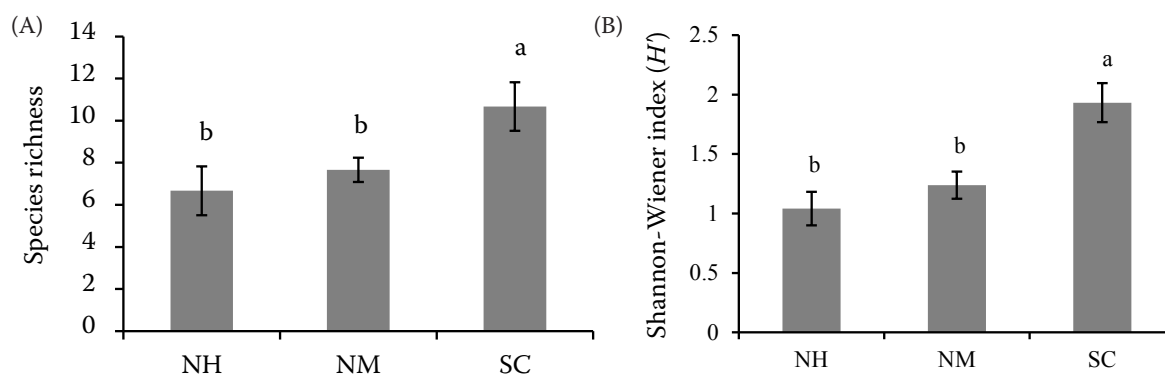


Figure 3. Species richness (A) and Shannon-Wiener index, (B) of arbuscular mycorrhizal fungi (AMF) in three types of soil management (SM) citrus orchards. Bars with the same letter are not significantly different ($P < 0.05$); NH – no-tillage; NM – straw mulching; SC – sod culture

In the present study, the higher AMF diversity in SC might be advantageous in improving various orchard ecology functions as different AMF species were highly variable in several functional traits (Thonar et al. 2011).

Influence of environmental variables on AMF community. RDA analysis showed that the coordinate from the first two ordination axes explained 80.6% (the first axis: 64.2% and the second 16.4%) of the variance, and the significance (Monte Carlo permutation tests) of all canonical axes was $P = 0.01$. In Figure 4, the angle between a vector and any axis is a measure of the degree of correlation of the variable with the axis. Therefore, the pH and C_{ox} along the first axis ($P = 0.024$) result in the most differences of AMF distribution in the present survey. The P_{Olsen} , AN and AK were positively correlated with the second axis, but the P_{Olsen} had the higher degree of correlation with AMF distribution. Tian et

al. (2009) also reported that pH, C_{ox} and P_{Olsen} had severe influences on the distribution of AMF community. The results indicated that the soil environment modification caused by different SM practices should be a major factor impacting the AMF spore communities in citrus orchards. Additionally, the relationship between an AMF species RA and any selected environmental factor was shown in Figure 4. For instance, the RA of *R. intraradices* was positively correlated with the C_{ox} and SM.

This study reveals differences in AM colonization, SN, HLD and fungal diversity in citrus orchards with three kinds of no-tillage SM practices. The results indicate that planting mycorrhizal grasses can greatly improve the AM developmental levels, fungal diversity and soil quality. Therefore, it is desirable to select SC as the best practice in hilly citrus orchard in order to enhance AMF benefits and promote sustainable agriculture.

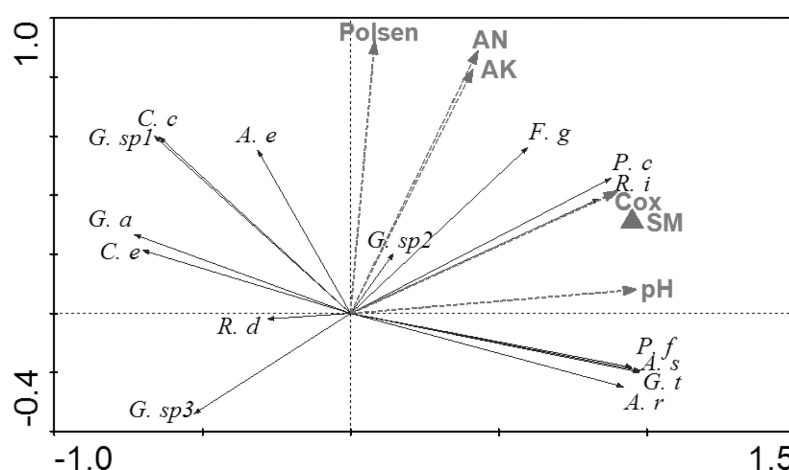


Figure 4. Ordination diagram from the RDA analysis of the relationship between the distribution of arbuscular mycorrhizal fungi (AMF) spore community and environmental variables in citrus orchards. The soil management (SM) is represented by solid triangle, the soil factor variables by dashed vectors, the AMF species by solid vectors. A. e – *A. excavate*; A. r – *A. rehmi*; A. s – *A. scrobiculata*; C. c – *C. claroideum*; C. e – *G. etunicatum*; F. g – *F. geosporum*; G. a – *G. aggregatum*; G. sp1 – *Glomus* sp1; G. sp2 – *Glomus* sp2; G. sp3 – *Glomus* sp3; G. t – *G. tenebrosus*; P. c – *P. chimonobambusae*; P. f – *P. franciscana*; R. d – *R. diaphanum*; R. i – *R. intraradices*

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Received on November 21, 2011

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