Research Article

Received: 9 April 2010 Revised: 1 September 2010 Accepted: 2 September 2010 Published online in Wiley Online Library: 24 September 2010

(wileyonlinelibrary.com) DOI 10.1002/jsfa.4180

No tillage affects the phosphorus status, isotopic composition and crop yield of *Phaseolus vulgaris* in a rain-fed farming system

María del Mar Alguacil,[a]* Antonio Roldán,[a] Jaime R Salinas-García[b] and José Ignacio Querejeta[a]

Abstract

BACKGROUND: Conservation tillage promotes the accretion of soil organic matter and often leads to improved soil fertility and moisture availability. However, few studies have looked at the physiological response of crop plants to different tillage practices. It was therefore hypothesised that measuring the nutrient concentrations and stable isotope composition (δ^{13}C, δ^{18}O, δ^{15}N) of shoots could help evaluate the physiological response of common bean (*Phaseolus vulgaris* L.) to different tillage treatments (no tillage (NT) and mouldboard ploughing (MP)) in a rain-fed farming system in northern Mexico.

RESULTS: NT significantly enhanced shoot phosphorus concentration in bean plants. Tillage exerted a negative effect on the extent of root colonisation (%) by arbuscular mycorrhizal fungi (AMF). Lower shoot δ^{18}O but unchanged δ^{13}C values in plants from the NT system suggest enhanced stomatal conductance but also enhanced photosynthetic rate, which overall resulted in unchanged water use efficiency. Bean plants in the NT system showed lower shoot δ^{15}N values, which suggests that a larger proportion of total plant nitrogen was obtained through atmospheric nitrogen fixation in this treatment.

CONCLUSION: Greater diversity of AMF soil communities and heavier colonisation of roots by AMF in the NT compared with the MP system appeared to contribute to improved crop nutrition, water relations and yield in this rain-fed agroecosystem.

Keywords: no tillage; δ^{13}C; δ^{18}O; δ^{15}N; *Phaseolus vulgaris*; rain-fed farming system

INTRODUCTION

The degradation of agroecosystems produces soil erosion and desertification problems worldwide.[1] Agricultural malpractice decreases soil organic matter content and the availability of water and nutrients, principally nitrogen (N) and phosphorus (P).[2–4] Therefore increased attention is being paid to the application of management practices that enhance the transformation and retention of soil organic matter to maintain the long-term sustainability of agroecosystems.[5,6] This is the case of conservation tillage systems such as no tillage, which promotes the accretion of soil organic matter. Numerous studies have demonstrated the beneficial effects of this conservation management system, which include improved soil fertility, reduced erosion, decreased weed problems and less need for chemical fertiliser use.[7–9] No tillage improves soil biochemical quality, with higher values of water-soluble carbon (C), soil organic matter[10] and soil microbial biomass C and N[3,4,11] in no-tilled compared with tilled soils. No tillage tends to improve soil physical properties as well, as higher aggregate stability and lower bulk density often result in enhanced soil water-holding capacity and moisture storage.[1,10,12–14] No tillage can also enhance the diversity of arbuscular mycorrhizal fungi (AMF), the abundance of AMF propagules and the extent of plant root colonisation (%) by AMF.[11,15]

AMF colonise the roots of the majority of agricultural crops and improve plant nutritional status and growth by enhancing the uptake of essential nutrients such as P.[16,17] AMF can also improve host plant water status, either indirectly by enhancing P nutrition and/or more directly through increased water uptake by extraradical hyphae.[17–19] Many studies have shown that increases in mycorrhizal colonisation often result in crop nutrition and yield improvement in different crops and agricultural management practices.[20–22]

Few studies have looked at the physiological response of crop plants to different tillage treatments.[23] Leaf gas exchange

* *Correspondence to: María del Mar Alguacil, CSIC, Centro de Edafología y Biología Aplicada del Segura, Department of Soil and Water Conservation, PO Box 164, Campus de Espinardo, E-30100 Murcia, Spain. E-mail: mmalguacil@cebas.csic.es*

a *CSIC, Centro de Edafología y Biología Aplicada del Segura, Department of Soil and Water Conservation, PO Box 164, Campus de Espinardo, E-30100 Murcia, Spain*

b *Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Centro Regional del Noreste, Campo Experimental Río Bravo, PO Box 172, Río Bravo, Tamaulipas 88900, Mexico*

measurements could help identify the physiological mechanisms responsible for crop yield variation between different tillage treatments, but this approach is labour- and time-demanding and only provides snapshot information at one point in time unless repeated measurements are conducted. Alternatively, analysing the stable isotope composition of shoots can provide insight into plant physiological status over longer periods (from months to growing seasons[24–27]). The C isotope composition ($\delta^{13}C$) of plant material provides a time-integrated index for evaluating intrinsic water use efficiency, defined as the ratio between photosynthetic C fixation and stomatal conductance.[24–28] The oxygen (O) isotope composition ($\delta^{18}O$) of shoots reflects the isotopic composition of soil water taken up by the plant, evaporative and diffusional effects in transpiring leaves and isotopic exchange between O atoms in organic molecules and plant water.[29] Recent studies have demonstrated that leaf $\delta^{18}O$ can provide a time-integrated measure of plant stomatal conductance when other sources of variation (mainly source water $\delta^{18}O$) are negligible.[29–32] Further, the joint analysis of $\delta^{13}C$ and $\delta^{18}O$ in shoots can help separate the independent effects of photosynthetic rate and stomatal conductance on $\delta^{13}C$, because $\delta^{18}O$ shares dependence on stomatal conductance with $\delta^{13}C$ but is unaffected by C fixation rate.[33–35] The N isotope composition ($\delta^{15}N$) of shoots reflects the net effect of a diverse array of factors, including the isotopic signature of soil N sources, plant mycorrhizal status, temporal and spatial variation in N availability and changes in plant demand.[24,36]

A number of recent studies have shown that analysis of plant isotopic composition can shed light on the physiological response of non-crop species to a wide range of factors, including mycorrhizal inoculation and planting method in afforestation schemes[25–27,37] and competition intensity in natural plant communities.[38] We therefore hypothesised that measuring the nutrient concentrations (N, P, potassium (K)) and stable isotope composition ($\delta^{13}C$, $\delta^{18}O$, $\delta^{15}N$) of shoots could help assess the physiological response of common bean (*Phaseolus vulgaris* L.) to different tillage treatments in a rain-fed farming system.

MATERIALS AND METHODS

Site description

This research was conducted at the Río Bravo experimental site in northern Tamaulipas, Mexico (25° 57′ N, 98° 01′ W). The dominant soil type is Vertisol,[39] developed from alluvial sediments with a clay texture (28% sand, 41% clay and 31% silt by weight), 12 g kg^{-1} organic matter and a pH of 7.8 (1 : 2 soil/water). The climate of the region is classified as warm subtropical, with hot, wet summers and dry winters. The annual temperature averages 23 °C and the annual rainfall averages 635 mm. The topography of the area is mainly flat, and slopes do not exceed 3%. The climax vegetation of this area has almost disappeared owing to agriculture and is currently represented by patches of shrub species such as *Prosopis juliflora* and *Acacia farnesiana* and halophytic pasture.

Experimental design and layout

The experiment was conducted using a full randomised design with three replication plots. Plots measured 22.4 m by 52 m. Subplots were subjected to two different tillage treatments: mouldboard ploughing (disking stalks after harvest, followed by mouldboard ploughing and discing, then building the rows) and no tillage (shredding stalks after harvest and spraying glyphosate (1.5 L ha^{-1}) and 2,4-D amine (1.5 L ha^{-1}) as needed for weed control). The two cropping systems were established in 2000, as described previously.[3] Common bean was planted in late January and harvested in the first half of June each year from 2000 to 2005.

Sampling and laboratory procedures

All samples were collected in the second half of May 2005. Thirty plants from each tillage treatment (ten per replication plot) were sampled and taken to the laboratory. Plant biomass was dried (60 °C for 48 h), weighed and then converted to kg ha^{-1}. Oven-dried plant tissues were finely ground before chemical analysis. Only new shoots and leaves were used for nutrient and isotopic determinations. After digestion of the leaf samples in nitric/perchloric acid (5 : 3) for 6 h at 210 °C, foliar P was measured by colorimetry, foliar N was determined by the Kjeldahl method and foliar K was estimated by flame photometry as described by Caravaca *et al.*[40]

All isotopic analyses were conducted at the Center for Stable Isotope Biogeochemistry, University of California, Berkeley, CA, USA. $\delta^{13}C$ and $\delta^{15}N$ analyses were performed by elemental analyser/continuous flow isotope ratio mass spectrometry using an ANCA/SL elemental analyser (Europa Scientific, Crewe, UK) coupled with a PDZ Europa Scientific 20/20 mass spectrometer. $\delta^{18}O$ analysis was conducted in a Finnigan MAT Delta PlusXL (Finnigan MAT, Bremen, Germany) following the method of Farquhar *et al.*[41] All isotope ratios are expressed in δ notation, where the isotopic composition of a material relative to that of an internationally accepted reference standard is given by $\delta^{xx}E$ (‰) $= [(R_{sample}/R_{standard}) - 1] \times 1000$, where R is the molecular ratio of heavy to light isotope forms.[24] The standard used for $\delta^{13}C$ is PeeDee Belemnite (V-PDB), the standard used for $\delta^{15}N$ is atmospheric air and the standard used for $\delta^{18}O$ is Vienna standard mean ocean water (V-SMOW). Long-term (3+ years) external precisions for C and O isotope analyses are 0.17 and 0.23‰respectively.

The extent of mycorrhizal root colonisation (%) was estimated by visual observation of fungal colonisation after clearing washed roots (0.2 g per plant) in 100 mL L^{-1} KOH and staining with 0.5 mL L^{-1} trypan blue in lactic acid according to Phillips and Hayman.[42] The extent of mycorrhizal colonisation was calculated by the gridline intersect method.[43]

Statistical analysis

Treatment effects on measured variables were determined by analysis of variance, and comparisons among treatment means were made using a least significant difference (LSD) test at $P < 0.05$. Correlation coefficients among measured variables were calculated by Pearson's correlation analysis. Statistical procedures were carried out with the software package SPSS for Windows 15.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

No tillage (NT) significantly enhanced shoot P concentration in bean plants compared with mouldboard ploughing (MP) (Table 1). Plant biomass and crop yield were also marginally higher ($P < 0.10$) in the NT management system. These results may have been partly due to the beneficial effects of greater root colonisation by native AMF under NT than under MP (Table 1). Across tillage treatments we found a strong positive correlation between % AMF colonisation and shoot P concentration (Pearson's $r = 0.901$, $P < 0.05$). Previous studies conducted under different agricultural management practices[20,21,44] have shown that crop yield is usually

Table 1. Shoot nutrient concentrations, extent of AM fungal colonisation in roots, dry plant biomass and crop yield of bean plants in different tillage systems

Parameter	Mouldboard ploughing	No tillage	*P* value
Nitrogen (mg g^{-1}) ($n = 30$)	17.1 ± 0.4	16.2 ± 0.6	NS
Phosphorus (mg g^{-1}) ($n = 30$)	0.82 ± 0.03	1.02 ± 0.02	0.023
Potassium (mg g^{-1}) ($n = 30$)	17.4 ± 0.4	19.3 ± 0.6	NS
AM root colonisation (%) ($n = 30$)	36 ± 1	42 ± 1	0.047
Dry plant biomass (kg ha^{-1}) ($n = 3$)	791 ± 17	889 ± 46	0.098
Bean production (kg ha^{-1}) ($n = 3$)	392 ± 8	440 ± 23	0.096

Values are mean ± standard error. NS, non-significant difference between tillage systems as determined by LSD test.

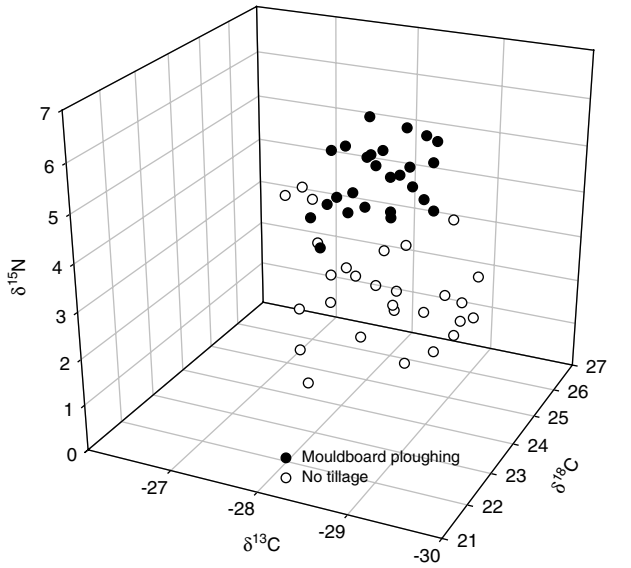

Figure 1. Three-dimensional representation of shoot carbon, nitrogen and oxygen stable isotope composition of bean plants grown under two different tillage treatments, i.e. mouldboard ploughing and no tillage ($n = 30$ plants for each tillage treatment).

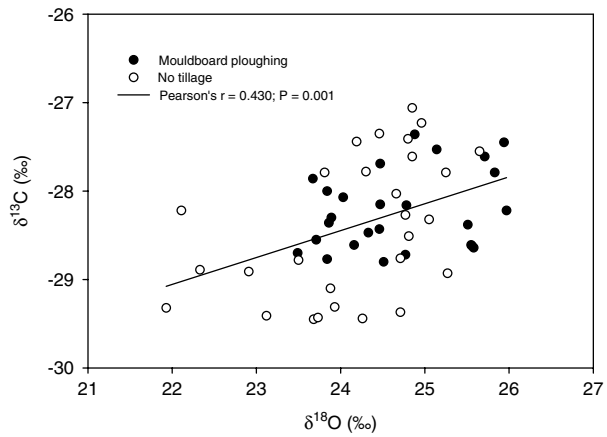

Figure 2. Positive relationship between shoot $\delta^{18}O$ and $\delta^{13}C$ across tillage treatments ($n = 60$).

enhanced by increased mycorrhizal colonisation, independently of the soil management practice used. Meta-analysis studies have shown that high mycorrhizal colonisation generally leads to increased plant P uptake in soils where reduced disturbance was applied.[21] In accordance with previous studies,[44,45] we found that tillage (MP) had negative effects on mycorrhizal colonisation and AMF diversity.[15] This could be due to the fact that tillage breaks up the native AMF hyphal network, which acts as an extension of host plant roots and as a direct link between roots and soil nutrient reserves. Thus tillage can sometimes cause a significant reduction in both mycorrhizal colonisation of roots and total P uptake by plants.[46] In contrast, Lekberg *et al.*[22] did not find a significant decrease in AMF abundance in soils subjected to tillage.

The greater extent of AMF root colonisation in the NT system is consistent with the results of a previous study reporting higher diversity of AMF types under NT than under MP in the same experimental plots.[15] Only two distinct AMF types were found under MP, while four distinct AMF types were found under NT. Of these AMF types, one was exclusive to the MP system (identified as *Glomus intraradices*), whereas three AMF types were exclusive to the NT system. The three fungal types exclusive to the NT treatment were found for the first time and could be novel AMF species. Differences in the taxonomic composition of the AMF community between the MP and NT treatments likely influenced the physiological response of *P. vulgaris* to each cropping system. It is increasingly recognised that AMF species can differ widely in the physiological and growth responses that they induce in their host plants.[25,37,47]

Shoot $\delta^{13}C$, $\delta^{18}O$ and $\delta^{15}N$ spanned a wider range of values in plants grown under NT than in those grown under MP (Figs 1 and 2). Plants grown under NT showed marginally lower shoot $\delta^{18}O$ values than those grown under MP (24.14 ± 0.18 *vs* 24.62 ± 0.15‰, $P < 0.086$), whereas shoot $\delta^{13}C$ was unaffected by tillage treatment (−28.23 ± 0.8‰in MP *vs* −28.40 ± 0.14‰in NT, $P = 0.326$). Shoot $\delta^{18}O$ provides a time-integrated measure of stomatal conductance and transpiration rate during the growing season,[29] such that a decrease in $\delta^{18}O$ indicates an increase in stomatal conductance.[31] $\delta^{13}C$ in plant biomass depends on the ratio between photosynthetic rate and stomatal conductance and provides a proxy index of intrinsic water use efficiency.[28] Lower shoot $\delta^{18}O$ but unchanged $\delta^{13}C$ values in plants from the NT system suggest enhanced stomatal conductance but also enhanced photosynthetic rate, which overall resulted in unchanged water use efficiency and $\delta^{13}C$.[33,34] The improved P status of plants under the NT system likely stimulated photosynthetic rate in addition to stomatal conductance.[25,37] This interpretation of isotopic data is supported by marginally ($P = 0.096$) higher crop biomass and yield in the NT system. Further detailed studies involving leaf gas exchange measurements in the field will be necessary to fully confirm this interpretation.

The $\delta^{18}O$ of plant tissue is influenced by the isotopic signature of source water used by the plant,[29] so differences in shoot $\delta^{18}O$ between the two cropping systems could have been determined by potential differences in source water $\delta^{18}O$. The crop residues remaining on the soil surface in NT systems have been shown to reduce water evaporation,[7] which might have contributed to less isotopically enriched soil water. However, it is important to emphasise that shoot $\delta^{18}O$ was positively correlated with

shoot $\delta^{13}C$ across management systems (Fig. 2), indicating that both parameters were largely under stomatal control.[34] This positive correlation between $\delta^{18}O$ and $\delta^{13}C$ strongly suggests that decreased shoot $\delta^{18}O$ in the NT system indicates enhanced stomatal conductance rather than (or in addition to) isotopically depleted source water $\delta^{18}O$ (Fig. 1).

Increased organic matter content and soil aggregation in NT systems improve soil water-holding capacity, thus leading to enhanced moisture availability for plants.[3,4,7,11,48] A positive mycorrhizal effect on plant water relations may have also contributed to enhanced biomass production and crop yield in the NT treatment. Higher AMF colonisation rates can lead to greater hyphal water uptake and transport to the host plant, increased root hydraulic conductivity and improved plant stomatal regulation and osmotic adjustment.[17,18] Further, AMF modification of rhizosphere soil may be as or more important than direct AMF effects on plant water relations.[49] The AMF extraradical mycelium can significantly enhance soil moisture retention capacity, since mycorrhizal soil often contains more water-stable aggregates than non-mycorrhizal soil.[49,50]

Bean plants in the NT system showed significantly ($P < 0.001$) lower shoot $\delta^{15}N$ values than those in the MP system (2.75 ± 0.14 *vs* 4.87 ± 0.16‰). Shoot $\delta^{15}N$ values closer to 0‰in the NT system suggest that a greater proportion of total plant N may have been obtained through atmospheric N_2 fixation, as soil N is typically enriched in ^{15}N compared with atmospheric N_2 (the $\delta^{15}N$ of atmospheric N_2 is by definition 0.0‰[24,51]). Several studies have reported greater diversity of soil rhizobial communities and higher biological N_2 fixation rates under NT compared with conventional tillage.[52–54] Further, we found that % AMF colonisation was negatively correlated with $\delta^{15}N$ (Pearson's $r = -0.816$, $P < 0.05$), as the more heavily colonised plants in the NT treatment had $\delta^{15}N$ values closer to 0‰. The well-known synergistic interaction between the rhizobial and AMF symbioses usually leads to greatly enhanced N_2 fixation rates in mycorrhizal legumes,[55–57] which may have contributed to greater fixation of atmospheric N_2 and therefore shoot $\delta^{15}N$ values closer to 0‰in the NT system.

CONCLUSIONS

The joint measurement of shoot $\delta^{13}C$, $\delta^{18}O$, $\delta^{15}N$ and nutrient concentrations holds promise as a useful tool for investigating the physiological response of crops to different tillage treatments. Greater diversity of AMF soil communities and heavier colonisation of roots by AMF in NT compared with conventional tillage systems may contribute to improved crop nutrition, water relations, growth and yield in rain-fed farming systems.

ACKNOWLEDGEMENT

MM Alguacil was supported by the Juan de la Cierva programme (Ministerio de Educación y Ciencia, Spain).

REFERENCES

1 Lal R, Minimum tillage systems, in *Subsoil Management Techniques*, ed. by Jayawardane NS and Stewart DA. Lewis, Boca Raton, FL, pp. 1–33 (1995).
2 Bayer C, Martin-Neto L, Mielniczuk J, Pillon CN and Sangoi L, Changes in soil organic matter fractions under subtropical no-till cropping systems. *Soil Sci Soc Am J* **65**:1473–1478 (2001).
3 Roldán A, Salinas-García JR, Alguacil MM and Caravaca F, Changes in soil enzyme activity, fertility, aggregation and C sequestration mediated by conservation tillage practices and water regime in a maize field. *Appl Soil Ecol* **30**:11–20 (2005).
4 Roldán A, Salinas-García JR, Alguacil MM, Díaz E and Caravaca F, Soil enzyme activities suggest advantages of conservation tillage practices in sorghum cultivation under subtropical conditions. *Geoderma* **129**:178–185 (2005).
5 Paustian K, Six J, Elliott ET and Hunt HW, Management options for reducing CO_2 emissions from agricultural soils. *Biogeochemistry* **48**:147–163 (2000).
6 Roldán A, Caravaca F, Hernández MT, García C, Sánchez-Brito C, Velásquez M, *et al*, No tillage, crop residue additions, and legume cover cropping effects on soil quality characteristics under maize in Patzcuaro watershed (Mexico). *Soil Till Res* **72**:65–73 (2003).
7 Tiscareño M, Báez-González AD, Velásquez-Valle M, Potter KN, Stone JJ, Tapia-Vargas M, *et al*, Agricultural research for watershed restoration in central Mexico. *J Soil Water Conserv* **54**:686–692 (1999).
8 Salinas-García JR, Matocha JE and Hons FM, Long-term tillage and nitrogen fertilization effects on soil properties of an Alfisol under dryland corn/cotton production. *Soil Till Res* **42**:79–93 (1997).
9 Salinas-García JR, Velázquez-García JJ, Gallardo-Valdez M, Díaz-Mederos P, Caballero-Hernández F, Tapia-Vargas LM, *et al*, Tillage effects on microbial biomass and nutrient distribution in soils under rain-fed corn production in central-western Mexico. *Soil Till Res* **66**:143–152 (2002).
10 He J, Kuhn NJ, Zhang XM, Zhang XR and Li HW, Effects of 10 years of conservation tillage on soil properties and productivity in the farming–pastoral ecotone of Inner Mongolia, China. *Soil Use Manag* **25**:201–209 (2009).
11 Roldán A, Salinas-García JR, Alguacil MM and Caravaca F, Changes in soil sustainability indicators following conservation tillage practices under subtropical maize and bean crops. *Soil Till Res* **93**:273–282 (2007).
12 Larney FJ and Lindwall CW, Rotation and tillage effects on available soil water for winter wheat in a semi-arid environment. *Soil Till Res* **36**:111–127 (1995).
13 Larney FJ, Janzen HH, Smith EG and Anderson DW, Dryland agriculture on the Canadian Prairies: current issues and future challenges, in *Challenges and Strategies for Dryland Agriculture*, ed. by Rao SC and Ryan J. American Society of Agronomy, Madison, WI, pp. 113–138 (2004).
14 Lenssen AW, Johnson GD and Carlson GR, Cropping sequence and tillage system influences annual crop production and water use in semiarid Montana, USA. *Field Crop Res* **100**:32–43 (2007).
15 Alguacil MM, Lumini E, Roldán A, Salinas-García JR, Bonfante P and Bianciotto V, The impact of tillage practices on arbuscular mycorrhizal fungal diversity in subtropical crops. *Ecol Appl* **18**:527–536 (2008).
16 Smith SE and Read DJ, *Mycorrhizal Symbiosis*. Academic Press, London (1997).
17 Augé RM, Water relations, drought and vesicular–arbuscular mycorrhizal symbiosis. *Mycorrhiza* **11**:3–42 (2001).
18 Ruiz-Lozano JM and Azcón R, Hyphal contribution to water uptake in mycorrhizal plants as affected by the fungal species and water status. *Physiol Plant* **95**:472–478 (1995).
19 Ruiz-Lozano JM, Azcón R and Gómez M, Effects of arbuscular mycorrhizal *Glomus* species on drought tolerance: physiological and nutritional plant responses. *Appl Environ Microbiol* **61**:456–460 (1995).
20 McGonigle TP, A numerical analysis of published field trials with vesicular–arbuscular mycorrhizal fungi. *Funct Ecol* **2**:473–478 (1988).
21 Lekberg Y and Koide RT, Is plant performance limited by abundance of arbuscular mycorrhizal fungi? A meta-analysis of studies published between 1988 and 2003. *New Phytol* **168**:189–204 (2005).
22 Lekberg Y, Koide RT and Twomlow SJ, Effect of agricultural management practices on arbuscular mycorrhizal fungal abundance in low-input cropping systems of southern Africa: a case study from Zimbabwe. *Biol Fertil Soils* **44**:917–923 (2008).
23 Walley FL, Matus AG, Lafond P and van Kessel C, Water-use efficiency and carbon isotopic composition in reduced tillage systems. *Soil Sci Soc Am J* **63**:356–361 (1999).
24 Dawson TE, Mambelli S, Plamboeck AH, Templer PH and Tu KP, Stable isotopes in plant ecology. *Annu Rev Ecol Evol* **S33**:507–559 (2002).
25 Querejeta JI, Allen MF, Caravaca F and Roldán A, Differential modulation of host plant $\delta^{13}C$ and $\delta^{18}O$ by native and non-native

arbuscular mycorrhizal fungi in a semiarid environment. *New Phytol* **169**:379–387 (2006).
26 Querejeta JI, Allen MF, Alguacil MM and Roldán A, Plant isotopic composition provides insight into mechanisms underlying growth stimulation by AM fungi in a semiarid environment. *Funct Plant Biol* **34**:683–691 (2007).
27 Querejeta JI, Barberá GG, Granados A and Castillo VM, Afforestation method affects the isotopic composition of planted *Pinus halepensis* in a semiarid region of Spain. *Forest Ecol Manag* **254**:56–64 (2008).
28 Farquhar GD, Ehleringer JR and Hubick KT, Carbon isotope discrimination and photosynthesis. *Annu Rev Plant Physiol Plant Mol Biol* **40**:503–537 (1989).
29 Barbour MM, Stable oxygen isotope composition of plant tissue: a review. *Funct Plant Biol* **34**:83–94 (2007).
30 Barbour MM and Farquhar GD, Relative humidity- and ABA-induced variation in carbon and oxygen isotope ratios of cotton leaves. *Plant Cell Environ* **23**:473–485 (2000).
31 Barbour MM, Fischer RA, Sayre KD and Farquhar GD, Oxygen isotope ratio of leaf and grain material correlates with stomatal conductance and grain yield in irrigated wheat. *Aust J Plant Physiol* **27**:625–637 (2000).
32 Farquhar GD, Cernusak LA and Barnes B, Heavy water fractionation during transpiration. *Plant Physiol* **143**:11–18 (2007).
33 Scheidegger Y, Saurer M, Bahn M and Siegwolf R, Linking stable oxygen and carbon isotopes with stomatal conductance and photosynthetic capacity: a conceptual model. *Oecologia* **125**:350–357 (2000).
34 Grams TEE, Kozovitz AR, Häberle K-H, Matyssek R and Dawson TE, Combining $\delta^{13}C$ and $\delta^{18}O$ analyses to unravel competition, CO_2 and O_3 effects on the physiological performance of different-aged trees. *Plant Cell Environ* **30**:1023–1034 (2007).
35 Sullivan PF and Welker JM, Variation in leaf physiology of *Salix arctica* within and across ecosystems in the High Arctic: test of a dual delta C-13 and delta O-18 conceptual model. *Oecologia* **151**:372–386 (2007).
36 Högberg P, ^{15}N natural abundance in soil–plant systems. *New Phytol* **137**:179–203 (1997).
37 Querejeta JI, Barea JM, Allen MF, Caravaca F and Roldán A, Differential response of $\delta^{13}C$ and water use efficiency to arbuscular mycorrhizal infection in two aridland woody plant species. *Oecologia* **135**:510–515 (2003).
38 Ramírez DA, Querejeta JI and Bellot J, Bulk leaf $\delta^{18}O$ and $\delta^{13}C$ reflect the intensity of intraspecific competition for water in a semi-arid tussock grassland. *Plant Cell Environ* **32**:1346–1356 (2009). DOI: 10.1111/j.1365–3040.2009.02002.x.
39 FAO, *Soil Map of the World: Revised Legend*. Food and Agriculture Organisation of the United Nations, Rome (1988).
40 Caravaca F, Figueroa D, Alguacil MM and Roldán A, Application of composted urban residue enhanced the performance of afforested shrub species in a degraded semiarid land. *Bioresour Technol* **90**:65–70 (2003).
41 Farquhar GD, Henry BK and Styles JM, A rapid on-line technique for determination of oxygen isotope composition of nitrogen-containing organic matter and water. *Rapid Commun Mass Spectrom* **11**:1554–1560 (1997).
42 Phillips JM and Hayman DS, Improved procedures for clearing roots and staining parasitic and vesicular–arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans Br Mycol Soc* **55**:158–161 (1970).
43 Giovannetti M and Mosse B, An evaluation of techniques for measuring vesicular–arbuscular mycorrhizal infection in roots. *New Phytol* **84**:489–499 (1980).
44 Kabir Z, O'Halloran IP, Fyles JW and Hamel C, Seasonal changes of arbuscular mycorrhizal fungi as affected by tillage practices and fertilization: hyphal density and mycorrhizal root colonization. *Plant Soil* **192**:285–293 (1997).
45 McGonigle TP and Miller MH, Mycorrhizal development and phosphorus absorption in maize under conventional and reduced tillage. *Soil Sci Soc Am J* **57**:1002–1006 (1993).
46 McGonigle TP and Miller MH, Mycorrhizae, phosphorus absorption, and yield of maize in response to tillage. *Soil Sci Soc Am J* **60**:1856–1861 (1996).
47 Klironomos JN, Variation in plant response to native and exotic arbuscular mycorrhizal fungi. *Ecology* **84**:2292–2301 (2003).
48 Six J, Elliott ET and Paustian K, Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. *Soil Biol Biochem* **32**:2099–2103 (2000).
49 Augé RM, Stodola AJW, Tims JE and Saxton AM, Moisture retention properties of a mycorrhizal soil. *Plant Soil* **230**:87–97 (2001).
50 Rillig MC and Mummey DL, (Tansley review) Mycorrhizas and soil structure. *New Phytol* **171**:41–53 (2006).
51 Shearer G, Kohl DH, Virginia RA, Bryan BA, Skeeters JL, Nielsen ET, *et al*, Estimates of N_2 fixation from variation in the natural abundance of N^{15} in Sonoran desert ecosystems. *Oecologia* **56**:365–373 (1983).
52 Ferreira MC, Andrade DS, Chueire LMO, Takemura SM and Hungria M, Tillage method and crop rotation effects on the population sizes and diversity of bradyrhizobia nodulating soybean. *Soil Biol Biochem* **32**:627–637 (2000).
53 Kaschuk G, Hungria M, Santos JCP and Berton-Junior JF, Differences in common bean rhizobial populations associated with soil tillage management in southern Brazil. *Soil Till Res* **87**:205–217 (2006).
54 Loureiro MDF, Kaschuk G, Alberton O and Hungria M, Soybean [*Glycine max* (L.) Merrill] rhizobial diversity in Brazilian oxisols under various soil, cropping, and inoculation managements. *Biol Fertil Soils* **43**:665–674 (2007).
55 Azcón-Aguilar C, Azcón R and Barea JM, Endomycorrhizal fungi and *Rhizobium* as biological fertilisers for *Medicago sativa* in normal cultivation. *Nature* **279**:325–327 (1979).
56 Azcón R, Rubio R and Barea JM, Selective interactions between different species of mycorrhizal fungi and *Rhizobium meliloti* strains, and their effects on growth, N_2-fixation (^{15}N) and nutrition of *Medicago sativa* L. *New Phytol* **117**:399–404 (1991).
57 Barea JM, Azcón-Aguilar C and Azcón R, Vesicular–arbuscular mycorrhiza improve both symbiotic N_2 fixation and N uptake from soil as assessed with a ^{15}N technique under field conditions. *New Phytol* **106**:717–725 (1987).

