

Below-ground interactions for sustainable cropping systems

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Summary

Key aspects of cropping systems are described in terms of their impacts on soil, water and air resources. The importance of microbial symbiotic relations with crop plants are briefly considered in the context of nutrient resource use efficiency and the resilience of plants to biotic and abiotic stress. It is argued that cropping systems need to include crops with fibrous root systems and legumes in the rotation to ensure sustainable use of resources. Three series of experiments are discussed that considered how the efficacy of rhizobia and indigenous arbuscular mycorrhizal fungi could be enhanced in sustainable cropping systems. Evidence is presented to demonstrate that there are potential benefits to crop production from improved management of soil resources through the adoption of reduced tillage practices coupled with suitable crop rotation and weed control.

Introduction

Cropping systems describe the temporal and spatial arrangements of crops together with the associated management of soil, water and vegetation. Although the aim of a cropping system may be to enhance the productivity of land per unit of input (Lal, 2003), it must be evaluated over several crop cycles to ensure sustainability of the resource base. Soil resource needs to be resilient to the forces of wind and water erosion and less susceptible to the loss of plant nutrients or transport of contaminants by leaching or in surface runoff. Water resources need to be protected from excessive exploitation and contamination, while volatilization of ammonia and the release of gases, such as nitrous oxide and methane, to the atmosphere must be minimized. The total amount and the dynamics of soil organic matter, normally expressed in terms of carbon (soil organic carbon, SOC), are critical for these requirements and to maintain biological activity. Plant roots contribute to soil organic matter both by their death and decay and the release of mucilage from their apical regions. During their existence roots undergo extension and turnover; the latter occurring particularly within the various categories of branch roots (Goss and Watson, 2003). Fibrous root systems, typically associated with grasses, tend to result in greater root-length densities (the length of root per unit volume of soil) than do tap roots, which in turn can be faster growing. Larger values of root-length density help to enmesh soil particles and enhance the resistance of aggregates to disruptive forces. Extraction of water by roots helps increase the strength of aggregates as cementing agents, present in or derived from root-secreted mucilage or

decaying plant material, are brought into closer contact with soil particles as water menisci retreat from larger pores of the soil into finer ones (Goss and Kay, 2005). Over the last century and a half huge increases in agricultural production have come from the breeding of crop varieties that perform better, particularly being more resistant to disease, the development of more efficient and reliable systems of drainage and irrigation to meet the need for water and aeration, and the use of synthetic fertilizers to provide sufficient nitrogen (N) and phosphorus (P). Leguminous crops, particularly forage species, can obtain more than 80% of their N requirement from the atmosphere by biological N fixation through their symbiosis with rhizobia (Table 1). Some of the N fixed can be passed to non-fixing crops, for example to grass in a mixed stand with alfalfa or clover (Ta and Faris, 1987a; b), and after the legume crop has been harvested or ploughed under (Ebelhar *et al.*, 1984; Sheaffer and Seguin, 2003).

Table 1. *Estimates of crop N derived from N₂-fixation by legumes*

Plant	N in crop (kg N ha ⁻¹)	Plant N derived from the atmosphere (%)
Peanut	37-206	22-92
Soybean	17-450	14-98
Cowpea	9-39	12-70
Alfalfa	51-386	46-92
Annual medics	100-200	79-86
White clover	45-291	62-93
Red clover	15-373	35-87

The effectiveness of biological N fixation in legumes can be greatly enhanced if arbuscular mycorrhizal fungi (AMF) also colonize the roots; with the impact being greater if that occurs at an early stage of seedling development (Goss and de Varennes, 2002; de Varennes and Goss, 2007). There is evidence that some N may pass directly between fixing and non-fixing plants in a mixed stand through AMF (Haystead *et al.*, 1988; Frey and Schüepp, 1993). Uptake of mineral N released by the turnover of roots and nodules of legumes appears to be the more important mechanism (Goss *et al.*, 2002; Haystead and Marriott, 1979). The role of AMF in supplying to their hosts nutrients that have limited mobility in soil, such as P and Zn, is well established. Direct uptake of P by roots from the soil can be so down-regulated in the presence of AMF that the only supply is through the fungal hyphae (Smith *et al.*, 2004). Govindarajulu *et al.* (2005) showed that N can also move as ammonium ions (NH₄⁺) from the fungi into host root cells. Mycorrhizas confer further benefits to the host crop, including reduced uptake of toxic metals (Arines and Vilariño 1989; Bethlenfalvay and Franson 1989), improved tolerance to drought (Augé 2004; Cho *et al.* 2006) and greater resistance to soil-borne pathogens (Harrier and Watson, 2004). Mycorrhizal fungi also contribute importantly to development and maintenance of soil structure (Goss and Kay, 2005). Cropping systems need to capture and exploit all the advantages

offered by both microbial symbioses. Ensuring the optimum rate of colonization of a new crop by both AMF and rhizobia should be an important objective of soil management options within the cropping system. Enhancing mycorrhizal associations can be achieved by inoculating with exotic strains or by promoting the activity of indigenous AMF (Bagyaraj, 1992). However, inoculation at the field scale requires such a large amount of material that it is considered impractical (Fitter *et al.*, 2011). The success of inoculation with AMF is also determined by their ability to compete with indigenous fungi (Gianinazzi-Pearson and Diem, 1982). Practices that could enhance the efficacy of indigenous AMF would be of considerable benefit. Colonization can be initiated by three types of AMF propagule: spores, extraradical hyphae and hyphae from colonised roots fragments. Runner hyphae from a well-developed extraradical mycelium are quicker to initiate colonisation in a new host than other sources of inoculum (Martins and Read, 1997), particularly when the number of viable spores is limited (Read *et al.*, 1976) or soil temperature is not optimal (Entry *et al.*, 2002). Intensive tillage of soil will disrupt extraradical mycelium networks and limit the opportunities for colonization (Jasper *et al.*, 1989). In contrast, adoption of tillage systems that minimise soil disturbance below the depth of seed placement can encourage persistence of the extraradical mycelium. In temperate climates better protection of the soil surface is afforded by fall sowing of crops that require vernalization before reproductive growth can begin. However, such crops are harvested much earlier than the corresponding spring-sown varieties so increasing the period between components of a rotation. Under Mediterranean conditions varieties that have no requirement for vernalization are commonly grown over winter, resulting in an even longer period between successive crops as sufficient precipitation is required before sowing to ensure plant establishment. But is the longevity of the extraradical mycelium adequate under hot dry conditions in the absence of living host plants? Can the roots of the weeds, which germinate with the first rains, form a living bridge for mycelium networks between successive crops in the rotation? What effect soil disturbance has on the colonization is also important in developing strategies to optimize the viability of the extraradical mycelium.

Methodology

In the controlled environment pot studies that are discussed below, the underlying common experimental approach was based on that of Fairchild and Miller (1988). Essentially two different inoculum potentials of indigenous AMF were established by growing a base crop, firstly in a 'pretreatment' cycle, then in two or more 'treatment' cycles. Pots were filled with field soil that had been fully disrupted in passing through a 4-8 mm sieve. The soil was tamped to a bulk density of about 1.2 Mg m^{-2} . At the end of the pretreatment cycle, pots were selected at random and for half the soil was removed as two or three layers, which were kept separate, and again passed through a sieve before being repacked into the same layers within the pot. Root material that had not passed through the sieve was cut into lengths of approximately 1 cm and returned to the soil layer from which they had been separated. These pots formed the 'Disturbed' treatment. The remaining pots formed the 'Undisturbed' treatment. At the end of each treatment cycle soil in the pots of the Disturbed treatment was again disrupted as described above. Establishment of contrasting AMF inoculum potentials was defined by better growth of the host crop in at least the last of these treatment cycles. Further consequences of the differential inoculum potential were then investigated on the growth and development of a 'test' crop, usually over a single cycle. The water content of the soil was maintained at the value of that from

a well-drained field. Nutrients were applied to ensure that plants of the last treatment cycle and the test crop showed no deficiency symptoms.

In the first series, clay pots of soil were buried in the field with their tops level with the soil surface in the Alentejo region of Portugal and left without water for the period from the harvest of wheat (*Triticum aestivum* L., cv. Coa) of the last treatment cycle (July) until October. Wheat was used as the test crop. Disruption of the soil in the disturbed treatment only occurred in the pretreatment and treatment cycles and was not carried out after the last of those cycles or as part of the preparation for planting the test crop. The initial cycles were each of 21 days and the test crop was sampled after 10, 21 and 35 days growth.

In the second study, maize (*Zea mays*, L.) was sown for the pretreatment and treatment cycles and soybean (*Glycine max* (L.) Merr.) was used as the test crop. Peat-based Rhizobium (*Bradyrhizobium japonicum* strain 532C) inoculum was placed at the bottom of a small hole formed by a piece of dowel and the pregerminated seed of the soybean was placed on top and the soil eased back to cover the seed. To investigate early colonization the test crop (*Glycine max* (L.) Merr. cv. Korada) was sampled after 10, 23 and 49 days. To investigate the significance of soil P, Ca(H₂PO₄)₂.H₂O was added to pots at the start of the treatment cycles to achieve four levels of amendment, 0, 20, 40, and 80 mg P kg soil⁻¹. These soybean plants (*Glycine max* (L.) Merr. cv. Evans) were sampled only at podfill (49 days).

For the third study, a mixture of Mediterranean weeds was grown in each pot during the pretreatment cycle for at least one month. The weeds selected were Persian ryegrass (*Lolium rigidum* Gaudin), wild oat (*Avena sterilis* L.) and littleseed canarygrass (*Phalaris minor* Retz.). For the ‘Undisturbed’ treatment the weeds were killed using herbicide while in the ‘Disturbed’ treatment shoots and roots were chopped and incorporated during soil disturbance. The wheat was sampled after 21 and 28 days.

Results

Despite a period of two months exposure to severe drying coupled with hot air temperatures and the absence of supporting plants, indigenous AMF were able to colonize the following wheat crop. The greater colonization was associated with the establishment of the crop in the absence of significant soil disturbance below the depth of seeding during the treatment cycles (Table 2).

Table 2. Effect of soil disturbance during treatment cycles on arbuscular and hyphal colonisation of roots in a wheat test crop, between 10 and 35 days after planting

Previous soil treatment	Days after emergence	Proportional AM colonisation	
		Hyphal	Arbuscular
Undisturbed	10	0.10 ^c	0.04 ^d
Disturbed		0.07 ^c	0.02 ^d
Undisturbed	21	0.19 ^b	0.11 ^c
Disturbed		0.10 ^c	0.06 ^d
Undisturbed	35	0.29 ^a	0.21 ^a
Disturbed		0.21 ^b	0.15 ^b

Values with the same letter within columns are not significantly different from each other (P = 0.05).

Assuming development of an intact extraradical mycelium in the disturbed treatment was restricted to the last treatment cycle, the difference in colonization must have been the result of a greater resilience of the mycelium that had been developed over the three or four cycles in the undisturbed treatment.

Table 3. *Early effect of soil disturbance on AMF colonization and nodule formation in soybean*

Soil treatment	Days after emergence	AMF colonization parameters			Nodules plant ⁻¹	
		Hyphae (%)	Arbuscules (%)	Vesicles (%)	Number	Dry weight (mg)
Undisturbed	10	70 ^a	56 ^a	0.8 ^a	14 ^a	-
Disturbed		17 ^b	14 ^b	0.0 ^b	8 ^b	-
Undisturbed	23	85 ^a	79 ^a	5.0 ^a	25 ^a	18 ^a
Disturbed		43 ^b	42 ^b	0.3 ^b	21 ^a	4 ^b

For each date, values in a column followed by the same letter are not significantly different as estimated by the t-test at $p < 0.05$.

The greater AMF inoculum potential associated with the undisturbed treatment resulted in faster formation of mycorrhizas in soybean (Table 3). However, by podfill roots were colonized by hyphae or arbuscules to about 80% or more and differences induced by soil disturbance in these parameters were small or not statistically (Table 4). In contrast, the frequency of vesicles in roots from the disturbed treatment was only about half that of plants grown in undisturbed soil (Table 4); consistent with there being faster colonization by AMF in the absence of disturbance. The addition of large applications of P fertilizer tended to reduce colonization rates (Table 4).

Table 4. *The effect of P fertilizer application and soil disturbance on AMF colonisation in soybean roots at podfill (R₅)*

Treatment	Soil disturbance		Phosphorus applied (P mg kg ⁻¹ soil)			
	Undisturbed	Disturbed	0	20	40	80
Hyphal	94 ^a	93 ^a	96 ^a	95 ^a	92 ^a	89 ^b
Arbuscular	79 ^a	78 ^b	85 ^a	81 ^{ab}	77 ^{bc}	72 ^c
Vesicular	15 ^a	8.9 ^b	14 ^a	13 ^a	12 ^a	7.9 ^b

Values followed by the same letter in the same row and under the same treatment are not significant at 5%

The faster colonization of the soybean roots by AMF was accompanied by earlier nodule formation (Table 3). When P levels in the soil were small the total weight of nodules from plants in disturbed soil was less than those from undisturbed soil although the number of nodules was about the same (Table 3). As P levels increased the number of nodules tended to increase, especially in undisturbed soil but differences in weight of nodules were greatly reduced (Data not shown). The proportion of N in the plant that was derived from the atmosphere was greater in undisturbed than disturbed soil (Tables 5 and 6) and was enhanced by the addition of P (Table 6).

Table 5. *Effect of soil disturbance on the N content, N₂fixation, and use of soil N by soybeans*

Soil treatment	Days after emergence	N concentration (g kg ⁻¹ air-dry soil)	N content (mg plant ⁻¹)	Ndfa (%)
Undisturbed	10	75 ^a	15 ^a	-
Disturbed		74 ^a	16 ^a	-
Undisturbed	23	49 ^a	28 ^a	-
Disturbed		46 ^b	25 ^a	-
Undisturbed	49	32 ^b	89 ^a	32 ^a
Disturbed		39 ^a	76 ^b	12 ^b

For each date, values in a column followed by the same letter are not significantly different as estimated by the t-test at p=0.05. Ndfa – N derived from the atmosphere

The rate of N fixation measured at podfill in disturbed soil (Table 6) was consistent with the values obtained from the differences between N in nodulating and non-nodulating soybean isolines, 2.4 and 0.5 mg N per plant day⁻¹ (Kadir, 1994).

Table 6. *Proportion of N derived from the atmosphere at podfill of soybean as determined by ¹⁵N dilution and the daily rate of fixation measured as plant N derived from ¹⁵N gas applied to roots*

P applied mgkg ⁻¹	Percentage of N derived from the atmosphere (by ¹⁵ N Dilution)			
	0	20	40	80
Undisturbed	34 ^{cA}	79 ^{bA}	86 ^{aA}	81 ^{aA}
Disturbed	28 ^{cB}	59 ^{bB}	76 ^{aB}	82 ^{aA}
Rate of N fixation in disturbed soil (mg N per plant day ⁻¹)	0.5 ^b	ND	ND	2.6 ^a

Values followed by the same letter (lower case) in the same row or under the same P treatment (upper case) are not significant at 5%. ND – Not determined

Arbuscular colonisation rate of wheat was enhanced 21 days after planting, in the treatment where weeds were controlled by herbicide rather than by soil disturbance. Enhanced AMF colonisation promoted early P acquisition and growth of the crop (Table 7). The method of weed control significantly affected wheat AM colonisation parameters after 14 and 21 days, with soil disturbance resulting in poorer AM colonisation (Table 8).

Table 7. *Effect of weed control method on wheat growth and AM colonisation parameters after 21 days*

Pretreatment	Shoot weight (g/pot)	P uptake (mg/pot)	Hyphal colonisation	Arbuscular colonisation
No Weeds	1.32 ^a	2.84 ^a	0.32 ^a	0.21 ^b
Weeds				
- Systemic herbicide	1.22 ^{ab}	2.70 ^a	0.39 ^a	0.28 ^a
- Soil Disturbed	1.02 ^b	2.03 ^b	0.22 ^b	0.18 ^b

For each measured parameter means followed by the same letter are not significantly different (p≤0.05).

Comparison of results for the ‘No weeds’ and ‘Systemic Herbicide treatments in Tables 7 and 8 suggest that the benefits of AM colonisation from extraradical mycelium associated with weed roots increased as that from other inoculum types decreased (difference between ‘No weeds treatments). The type of herbicide (contact or systemic) had no impact on colonisation of the wheat crop (Table 8).

Table 8. *Effect of weed control method on AM colonisation in wheat*

	Days after planting	With weeds prior to wheat planting			‘No weeds’
		Method of weed control			
		Systemic Herbicide	Contact Herbicide	Disturbance	
Hyphal colonisation	14	0.68 ^b	0.75 ^a	0.48 ^c	0.07 ^d
	21	0.84 ^a	0.77 ^a	0.46 ^b	0.09 ^c
Arbuscular colonisation	14	0.35 ^a	0.36 ^a	0.08 ^b	0.02 ^b
	21	0.40 ^a	0.33 ^a	0.14 ^b	0.02 ^c

For each measured parameter means followed by the same letter are not significantly different ($p \leq 0.05$).

Conclusions

Cropping systems need to include crops with fibrous root systems and legumes in the rotation to ensure sustainable use of resources. Coupled with tillage practices that limits soil disturbance to the depth of seed placement, crops can establish more effective symbioses with indigenous AMF. This can have important consequences in terms of greater resistance to drought and to soil-borne pathogens, improved utilization of P and other nutrients with slow mobility in soil but also the reduced availability of toxic elements. In addition, biological N fixation can be enhanced through interactions between AMF and rhizobia. Weeds may be a useful means of enhancing extraradical mycelium as a viable means of colonizing crops separated in time, particularly in warm dry periods.

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