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Rhizosphere biology and crop productivity—a review

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Abstract. There is great potential to use the wide genotypic and agronomically induced diversity of root systems and their exuded chemicals to influence rhizosphere biology to benefit crop production. Progress in the areas of pathogens and symbionts in this regard is clear. Further progress, especially related to interactions with non-pathogenic organisms, will rely on an appreciation of the properties of rhizospheres in the field: the spatial and temporal boundaries of these rhizospheres, and the effects of structural, chemical, and physical soil heterogeneity in which the roots and associated microorganisms exist and function. We consider the rhizosphere environment within Australian cropping systems in relation to the likely success of biological interventions, and provide 3 case studies that highlight the need to characterise the rhizosphere and the microbial interactions therein to capture agronomic benefits. New techniques are available that allow direct visualisation and quantification of rhizosphere processes in field conditions. These will no doubt help develop better genetic and agronomic approaches. Future success, as with those in the past, will rely on integrating interventions related to rhizosphere biology with other management constraints of specific farming systems.

Additional keywords: roots, exudates, soil, microorganisms, agronomy, genetics.

Introduction

With the notable exceptions of symbionts and pathogens, the study of soil biology in agriculture has historically dealt with the effect of agricultural practices on free-living organisms in the soil. There have been many studies of how agronomic practice and the variety of the seasons have affected the populations or activities of particular classes of soil organisms *per se* in the bulk soil. Data from such studies are difficult to convert into integrated information that can be used to improve the productivity of crops and the viability of cropping systems. What is important agriculturally is how the interactions between management and soil biology affect the performance of crops (Fig. 1). Roots are thus an integral component of the soil biology.

The study of soil biology considering roots as integral offers us insights into how we might improve agronomic practices and cultivars, i.e. how we might facilitate the innovative management of Australian farms that has enabled rises in productivity to keep ahead of the steadily falling terms of trade over the last few decades. There is, as yet, no substantial edifice of theory that can connect improved practice with the extraordinarily complex interactions between roots and organisms in the soil. Nevertheless, the idea of the rhizosphere, now 100 years old, has laid the foundation for such an edifice.

There is strong evidence that changes in agronomic practice have improved the productivity and sustainability of Australian farming systems by influencing (amongst other things) the soil biology. Processes that have been successfully harnessed include symbiotic nitrogen fixation, crop sequences to control disease and inhibitory organisms, and longer-term suppression of disease (Table 1). These operate around roots (either living, or as dead remnants) and in rhizospheres over wide spatial and temporal scales.

Evidence from fumigation experiments and from puzzling agronomic responses in field trials suggests that we can capitalise further on interactions between crop management and soil biology (Kirkegaard 1995; Bever 2003). An example of agronomic responses that implicate influential changes in soil biology are those associated with conservation farming practices. A particular puzzle has been that such practices invariably improve many attributes of soil that are associated with high fertility; structural stability, infiltration rates, faunal and microbiological activity, soil organic matter, are all typically increased. Yet farmers' evaluations of the crop performance in conservation farming, both in Australia and worldwide, have been highly variable (Kirkegaard 1995; Lyon *et al*. 2004). What is clear is that the apparently major improvements in soil properties do not always translate reliably into better crop yields. The range of possible contributory factors includes: increased pests and diseases; toxic chemicals arising from retained stubble; greater residual effects of herbicides; growth-inhibitory bacteria in the rhizosphere; inhibited root growth in the

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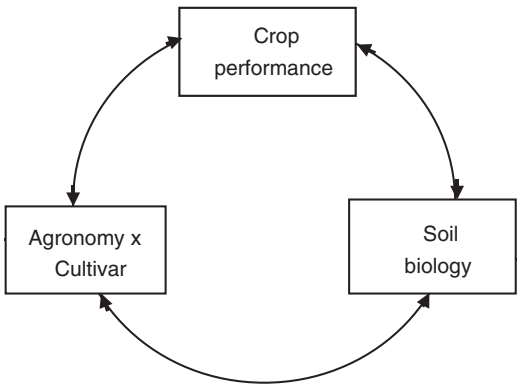


Fig. 1. Interactions among management, crop performance, and soil biology can be used to improve farming systems.

harder unploughed seed bed; inhibitory signals passing from roots to leaves when the roots are experiencing less than ideal soil conditions; and concentration of nutrients in the surface soil. Unravelling the underlying causes of crop response to changed agronomic practice such as this is difficult, but processes occurring within the rhizosphere are central.

This review aims to take stock of these various issues and the implications they might have for designing and interpreting agronomic experiments that aim to capture benefits from soil biology. It is organised into: (*1*) a section on the ecology of the rhizosphere, and especially of perennial rhizospheres — the niches that successive generations of roots occupy in unploughed soil, how their properties differ from those of bulk soil, and how diverse the properties of the roots are when growing in field soil; (*2*) three case studies of major agronomic effects that are becoming explicable in terms of interactions between roots and soil organisms; and (*3*) future directions including an analysis of diagnostic tools, and prospects for novel ways of harnessing biological activity in the soil to improve the performance of crops.

Ecology of the rhizosphere

The rhizosphere in laboratory and field

Most information about important processes in the rhizosphere comes from studies in controlled environments where roots are grown in simple uniform media, and organisms of interest are applied. Attempts to model rhizosphere processes have been instructive, but are too simplistic in their depictions of the rhizosphere to be agriculturally useful. They do highlight that compounds diffusing from roots stimulate bacterial growth (Newman and Watson 1977), that the water solubility of exudates and their pattern of exudation along a root affect how organisms grow (Darrah 1991), that wetter soil allows bacteria from the seed to migrate further along a root (Scott *et al*. 1995), and that bacterial death rates and predation can account for numbers of rhizosphere bacteria (Zelenev *et al*. 2000).

In the field, rhizospheres are much more complex than in the laboratory (McCully 1999). Field plants have more root types compared with those in the laboratory and a very broad range of chemicals is exuded from them and their associated organisms. The rhizospheres of field-grown roots experience large variation in environmental variables: in temperature, both diurnally and seasonally; in soil water, especially in the topsoil, which can range from air-dry to saturated in periods of hours to weeks; in structure, which offers niches at a range of scales, from continuous macropores that may enable rapid root extension in otherwise hard soil, to microcavities within soil aggregates that may protect resident bacteria from predation by larger organisms; in nutritional status, that may include concentrated pockets of nutrients, both organic and inorganic; in inocula, as in remnant roots that harbour large populations of microorganims; and in oxygen status, which may range from well-aerated to hypoxic within even a single aggregate. The inhabitants of the rhizosphere include microorganisms, macrofauna and insects. Bacteria generally are the most abundant (approx. 10^{10} cells/g of

Table 1. Agronomic practices well established in Australian cropping systems that exploit soil biological processes, and the time and spatial scales they occupy

Agronomic issue	Underlying soil biological processes	Time scale	Spatial scale
Nitrogen fixation	Infection of root hairs by rhizobia	Hours	μm to mm
	Nodule development and function	Days to weeks	mm
	Decomposition of N-containing tissue	Hours to years	mm to m
Crop sequence	Non-hosting of common diseases	Hours to months	mm to m
	Inoculum decline	Months to years	mm to m
Disease suppression	Bacteria increase on successive generations of dead roots	Years	μm to m
	Bacteria suppressive to fungi produce antifungal exudates in the crop rhizosphere	Hours to days	μm to mm

soil, or 10^6 cells/mm^3 rhizosphere biofilm), followed by fungi, protozoa, nematodes and insects (see Watt *et al*. 2006*b*; Doube and Brown 1998 for a review of rhizosphere macrofauna and insects). The diversity of each group is still being discovered (see *New methodologies* section below), and the abundance and diversity of organisms in the rhizosphere depend on the cropping environment, the plant species, the types of roots, their ages, and the chemicals exuded from them.

Wide spatial and temporal boundaries of the rhizosphere

The rhizosphere of a single root occupies a volume that extends from the root to an ill-defined position in the soil that depends on the diffusion of exudates and the stage of development and biochemistry of the roots (Hinsinger *et al*. 2005). Huisman (1982) used the distance within which fungi respond to root exudates to estimate rhizosphere width, and found it to be approximately 1.0 mm for most, but 5 and 12 mm for *Rhizoctonia* and *Gaemannomyces gramminis*, respectively. Bacteria tightly bound to the root, observed in solution under a microscope, sit within 0.03 mm of the root surface (Watt *et al*. 2006*a*). The characteristic time for exudates to diffuse and interact with a soil organism is the quotient of the distance squared and the diffusivity (see Watt *et al*. 2006*b*). Exudates exchange between roots and organisms much more quickly close to the root than further away, creating heterogeneity across the rhizosphere at a point in time. The rhizosphere can extend from the interior of the root along the length of hyphae of any fungi associated with that root. Within older roots, spaces left behind after cells have decomposed can harbour organisms (McCully 2001).

A rhizosphere is born when a root tip arrives in a volume of soil and ends when that piece of root decays (Jones *et al*. 2004). Over time, a root develops hairs and branch roots, and, in dicotyledon roots, secondary thickening. Depending on whether the crop is annual or perennial, the rhizosphere can persist for years. Once the root dies, it continues to harbour a succession of organisms that have important nutritional, disease, or other effects on new crop roots, as seen in rotations with legumes or canola (see below, *Case study 3*).

Shoots sense many rhizospheres of different ages from the various members of the root system. Cereal root systems consist of seminal roots, branch roots, and nodal roots. Dicotyledonous root systems have a tap root with successive orders of branch roots, and roots extending from the hypocotyl with their own branch roots. We know little about the infection dynamics of diseases, arbuscular mycorrhizal fungi (AMF), and other organisms, on these different types of roots. Sivasithamparam and Parker (1979) conducted one of the few studies of rhizosphere microorganisms on different root types. They found that seminal roots of wheat had more bacteria and actinomycetes than nodal roots both closely and loosely associated with the roots, whereas fungi were similar for both root types only in the closely associated fraction.

Bacterial populations change with root age. Populations on root tips differed from those at the root base of the seminal roots of wheat seedlings (Liljeroth *et al*. 1991). Sheathed and bare roots of field-grown maize had similar total numbers of bacteria, but the older bare roots were dominated by actinomycetes (Gochnauer *et al*. 1989). Specific compounds in exudates can be a major reason for differences in colonisation of different root types. McCully and Canny (1985) found that young and old regions of maize roots exude similar amounts of total carbon; however, the composition is different from each, and Liljeroth *et al*. (1991) found that bacteria from tips rather than those from the base, preferred citrate.

Generally, root systems occupy progressively deeper parts of the profile with time, so that the youngest regions of the axes are deepest. The various types of roots, and their extension rates and orientation, determine the extent of occupation of soil with depth, and the differences in soil structure, temperature, nutrients, and water at various depths influence how soil organisms interact dynamically with roots to affect crop growth. Designing temporal and spatial sampling strategies for agronomic experiments on, for example, biological inoculants, crop sequences, or the development of disease-suppressive soils, is difficult but crucially important.

Exudates and chemical signals

For nearly 200 years, scientists have known that roots exude chemicals that stimulate or suppress the activity of organisms in soil (Schroth and Hildebrand 1964). These organisms include microorganisms, seeds of root parasites, and other roots (Bertin *et al*. 2003; Bais *et al*. 2004). The central role of root exudates is particularly well recognised for fungi, some of which only break dormancy and germinate when exposed to such chemicals (see Akiyama *et al*. 2005, who showed that strigolactone induces branching of mycorrhizal fungi in culture).

Many rhizosphere chemicals are common constituents of root cells that have leaked from living roots or lysing, decomposing cells. Others are controlled metabolically by transport processes in the roots. Root exudates include protons and hydroxyl ions, water-soluble sugars such as sucrose and carboxylic anions, water-insoluble polysaccharides that become mucilage that protects roots and organisms, nitrogen-containing compounds such as amino acids (Merbach *et al*. 1999), and a very large range of secondary metabolites and signals. The net carbon (since, importantly, roots can take up rhizosphere carbon) released from roots ranges from 5 to 10% of net carbon fixed by the plant (reviewed in Farrar *et al*. 2003). These exudates support microbial activity in the rhizosphere. Modelling suggests that the growth of the microbial biomass in

the rhizosphere, supported by such carbon efflux, can, in itself, inhibit plant growth (~20% growth depression, Darrah 1998).

Rhizosphere organisms also contribute to the rhizosphere chemistry, releasing mineral nutrients from dead cells that can be taken up by roots, antibiotics and antifungal agents, phytotoxins (Gerhardson *et al*. 1985), and mucilages. One of the best characterised compounds of microbial origin is the antifungal toxin 2,4-diacetylphloroglucinol (DAPG) produced by *Pseudomonas* spp. that suppress pathogen growth (Keel *et al*. 1992). We focus here on mucilages, signals, and volatiles from roots.

Roots are covered by mucilage from roots and microorganisms (Greaves and Darbyshire 1972), which binds soil tightly when dry (Watt *et al*. 1993, 1994). Root mucilage is primarily produced by the root cap (border) cells, is left behind as the root grows forward, and contains complex polysaccharides with charged carboxyl groups, neutral sugars, proteins, and phenolics, depending on species (Miki *et al*. 1980). Root cap cells and their mucilage can selectively stimulate or inhibit rhizosphere bacteria (Gochnauer *et al*. 1990), and stimulate hyphal branching of the AMF, *Gigaspora gigantea* (Nagahashi and Douds 2004). The specific properties of mucilages are worthy of further research, in particular as sources of genetic variation between and within plant species.

Signals regulate numbers and activities of organisms, and root and shoot growth. The best characterised is the nod-factor-flavonoid exchange between legume root hairs and rhizobia that initiates nodule development (Brencic and Winans 2005). Specific signals regulating infection and invasion of roots by AMF or root disease organisms have not been identified. Acyl-homoserine lactone (AHLs) molecules produced by Gram-negative bacteria, regulate expression of genes within a group or 'quorum' of bacteria (Sharma *et al*. 2003). AHLs can be rapidly degraded in specific rhizosphere soils (Wang and Leadbetter 2005). Further, roots produce compounds that mimic the AHLs and therefore confuse communication between rhizosphere bacteria (Teplitski *et al*. 2000). Given this complexity the importance of AHLs to crop production, particularly in pores where successive roots are colonised (see below), remains to be demonstrated.

Signals of microbial origin can influence shoot processes, including AHLs that can stimulate transpiration (Joseph and Phillips 2003) and lumichrome and other rhizobia molecules that stimulate leaf expansion (Matiru and Dakora 2005). Kirkegaard *et al*. (1999*b*) found that wheat leaf extension was inhibited by *Rhizoctonia* in the absence of water or nutrient stress in these leaves, and suggested that the plant responded to signals from the roots induced by low levels of interaction with the fungus. Rhizosphere bacteria such as *Pseudomonas* spp. also cause slower leaf growth in the absence of invading or reducing growth of roots (M. Watt, unpublished data). Given the enormous variety of compounds in the rhizosphere, the best strategy may be simply to screen for genotypes that do not respond to negative signals from the rhizosphere by maintaining leaf growth in the presence of deleterious organisms.

Root exudates that move in the gaseous phase of soils include isothiocyanates (ITC) released from members of the *Brassica* genus including canola (Rumberger and Marschner 2003), and hydrogen gas released from the nodules as a by-product of nitrogen fixation in certain legumes (Dong *et al*. 2003). ITCs may modify the bacteria around canola roots (see *Case study 3*). Hydrogen injected into soil stimulates plant growth, notably that of wheat (Dong *et al*. 2003), but not if the soil is sterilised, suggesting that hydrogen promotes growth via soil organisms that stimulate plant growth. As with canola roots and ITCs, hydrogen effects depend on distances between nodules and new roots, and the stage of succession of organisms around nodules. These dynamics remain to be unravelled in the field to identify new agronomic practices to take advantage of legume hydrogen.

Manipulating exudates for agronomic advantage

Exudate regulation of rhizosphere organisms in the field is still largely unknown. Gaps in knowledge include: the fate of exudates in soil, sites of synthesis and transport in and out of the root, sensing and receptors within the plant, and the specificity of different compounds for root and organism responses. Research, by necessity, is still done in simple systems devoid of the physical, chemical, and biological complexities of field soil, which influence diffusion distances and longevity of exudates. Recently, a *Pseudomonas syringae* leaf pathogen was found to block the synthesis of an antibacterial compound released from the roots of *Arabidopsis* exposed to other strains of *Pseudomonas syringae* (Bais *et al*. 2005). Charcoal rendered this antibacterial compound inactive as, presumably, would natural soil. Exudates, however, remain a critical target for manipulating soil biology for agronomic benefit because different genotypes with different roots can be used to modify the soil chemistry (O'Connell *et al*. 1996; Rengel and Marschner 2005). Developing screens to study and select for plants with exudate-mediated microorganism interactions, in conditions relevant to farming systems, remains a challenge.

Characteristics of the rhizosphere in Australian cropping soils

Soil structure and successive generations of roots in soil cracks and macropores

Most Australian cropping soils are difficult for roots to penetrate. Untilled soils are hard and force roots to grow much slower than in cultivated soil (Watt *et al*. 2005). Hard pans of soil can form below the ploughed layer,

impeding roots and movement of soil organisms. Most subsoils are very dense, exceeding 1.6 g/cm^3 bulk density. When roots encounter hard soil, extension is restricted and apices have much shorter elongation zones and are distorted (Fig. 2*a*, *b*). Roots in hard soil are more heavily infected by *Fusarium* in bean and *Rhizoctonia* in wheat (Burke *et al*. 1980; Gill *et al*. 2004). Bacteria accumulate on the apices of wheat roots (cv. Janz) in untilled soil (Fig. 2*c*; Watt *et al*. 2003). *Case study 1* below outlines how interactions between soil hardness and inhibitory bacteria in the rhizosphere can explain lowered productivity of wheat in conservation farming systems. Increases in organisms around roots in hard soil seem due to an interaction between slower growth rate and more exudates (Watt *et al*. 2006*b*). For example, roots in hard soil release more root cap cells and mucilage (Iijima *et al*. 2000) that are carbon-rich substrates for bacteria.

Perhaps more important than uniform strength in soil is the presence of cracks and large pores (Cresswell and Kirkegaard 1995), within which roots are often constrained to grow (Fig. 2*d*), leading to variation in plant growth (Stirzaker *et al*. 1996). Often, previous roots have occupied these spaces, which become niches that successive generations occupy. The surrounding soil has more microbial biomass that consumes more substrates than that in the bulk soil (Pierret *et al*. 1999). These niches may be in the unploughed surface soil in conservation cropping systems, and in the undisturbed soil below the plough layer.

Direct contact between new roots and dead remnants from previous crops or weeds is substantial (Fig. 2*e*). At least half of new roots were in direct contact with dead roots of previous crops in direct-drilled soils in south-eastern NSW (Watt *et al*. 2005). These dead roots harboured as many bacteria as young, living wheat roots; however, many more were filamentous, such as actinomycetes (inset, Fig. 2*c*). Root-to-root contact alters the bacterial population on the young roots (Fig. 2*f*, *g*), favouring filamentous bacteria, with fewer *Pseudomonas* (Watt *et al*. 2006*a*).

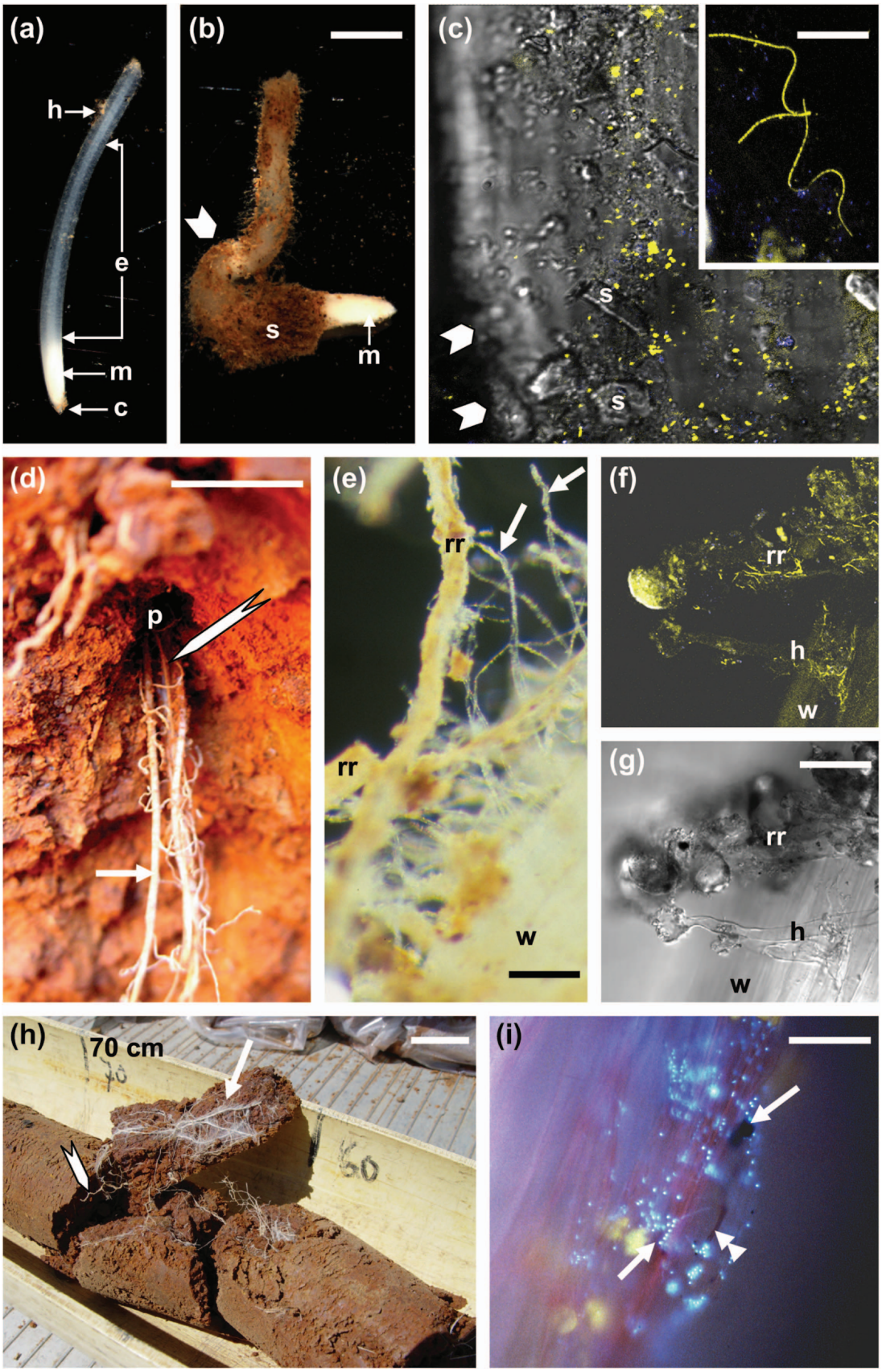
Organisms in cracks and pores may affect crops through nutrients, disease, symbiotic interactions, or other unknown effects on plant growth. Van Noordwijk *et al*. (1993) found more nitrogen mineralisation in cracks in soil with organic matter and new roots. McNeill *et al*. (1999) and Khan *et al*. (2001) found greater nitrogen in dead roots than in dead shoots of crops, and that this root nitrogen can be taken up by a subsequent wheat crop. Such pores can be richer in phosphorus (Pierret *et al*. 1999). Macrofauna and insects, and organisms transported by water that flows through spaces larger than 0.03 mm in diameter, would move quickly through pores. Subsoil roots as deep as 1.5 m are heavily colonised by bacteria and fungi (Fig. 2*h*, *i*), possibly carried from above through pores. Rhizosphere dynamics in pores may affect the worth of 'primer' plants that make holes in subsoils that can be used by subsequent crops (see final section).

Soil temperature

Roots and organisms encounter different temperatures depending on the depth of soil, time of year, and cropping region (Fig. 3). Most wheat in Australia sown in autumn has roots within the top 0.2 m where soil is on average 8°C in south-eastern New South Wales, and 13°C in south-eastern Queensland. During a season in south-eastern NSW, surface soil temperature varies by 20°C (Fig. 3*a*). Crops sown in April encounter soil 10°C warmer than if sown in June. The subsoil oscillates 10°C over the season, and in winter, is warmer than the surface soil. Temperature affects the rates of growth and metabolism of roots and organisms, which affect exudation, although the temperature optima for these root and organism processes can be different. Wheat root extension is more inhibited by *Pseudomonas* at 15°C than at 20°C (Elliott and Lynch 1985), and strawberry roots grown at cool temperatures (5 and 10°C) produce exudates that strongly stimulate germination and hyphal growth of *Rhizoctonia*, whereas roots grown at 20 and 30°C produced no such exudates (Husain and McKeen 1963).

Few studies have quantified the effect of temperature on individual root extension rates, and these few generally focus on young seminal axes. The extension rate of wheat seminal roots is 3.5 times slower at 7°C than at 15°C (S. Refshauge and M. Watt, unpublished), and that of maize is 2.8 times slower at 16°C than at 29°C (Pahlavian and Silk 1988). Cohen and Tadmor (1969; summarised here in Table 2) measured rates of descent of root systems, finding, as above, that young seedling root growth is reduced 3-fold when the temperature is reduced by half. The large variation across species was greater at cooler temperature (10-fold) than at warm (6-fold), and in surface soil compared with deeper (5-fold variation at both 10 and 20°C in deeper soil). Rates of descent in field environments also depend on other factors such as soil density, availability of continuous cracks and macropores, toxic elements, soil water content, and pathogenic organisms. Thus, rates in Table 2 may well overestimate rates in most field environments.

Microorganism activity also depends strongly on temperature. Of particular relevance to the rhizosphere is differential effects of temperature on growth rates of different types of soil organisms, and their relative ability to colonise root surfaces at different temperatures. Pietikäinen *et al*. (2005) found that both fungi and bacteria in soil grew quickest between 25 and 30°C, but that fungi were more inhibited above 30°C than bacteria, and that bacteria were more inhibited by cooler temperatures, regardless of soil type. Fungi to bacteria ratios may increase at cool temperatures, and decrease at warm in the rhizosphere. Leach (1947) showed that temperature differentially affected hyphal elongation rates, with *Pythium ultimum* growing 3.5 times faster than *Rhizoctonia solani* at 12°C, but only 1.7 times faster at 20°C. Agronomic effects of organisms can be influenced by soil temperature, as suggested by the inability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of AMF to enhance P uptake in wheat during the cool autumn conditions in southern Australia, as discussed in more detail in *Case study 2*.

Gilligan (1980) made direct measurements of hyphae on wheat roots grown in sand, using microscopy. The wheat pathogenic fungus, *Gaemannomyces graminis*, extended 2.8 times faster along roots at 19°C than at 10°C. Most fascinating was that at 19°C the fungus grew preferentially towards the root base, but that this directional growth was absent at the cooler temperature. The hyphae thus extended 3 times faster towards the root tip at 10°C. The author suggests that this directional growth is related to assimilate supply to the fungus, although the exact mechanisms by which this occurred remains unclear, particularly since root elongation rate (e.g. position of tip) was not tracked simultaneously. Gilligan's study shows how dynamics of growth between organisms and roots can be learned from direct, microscopic quantification, and that environmental factors relevant to the field, such as temperature, differentially affect the rates of the various processes. New techniques combining *in situ* tracking of organisms on roots, and microscopic image analysis (discussed below), offer opportunities to extend such work to a number of different organisms relevant to crop production.

Soil water and organism mobility

Soil water provides a film of water on surfaces within which organisms may propel themselves (e.g. with flagella), and can provide flows of water that can carry organisms substantial distances through large pores, if they do not stick strongly to soil surfaces (Camper *et al*. 1993). Boelens *et al*. (1994) used a motile and non-motile strain of a growth-promoting *Pseudomonas fluorescens* inoculant to show that motility did not influence the ability to colonise roots. Neither seed inoculation nor organism motility reliably helped distribute the inoculant. The flow of water plus extensive mixing of the inoculant through the tilled layer were much more effective in distributing the inoculant across the root system.

Moisture affects the diffusion of water-soluble chemicals in soil, and thence how quickly organisms receive a signal from a root. In wet soils, the rhizosphere is wide for organisms that respond to water-soluble compounds, and in dry soils the rhizosphere for such organisms is much closer to the root (Watt *et al*. 2006*b*). Very dry soil may cause organisms, including roots, to desiccate and die. Irrigated and flooded agricultural systems provide a special case where organisms, e.g. pathogens such as *Fusarium oxysporum* in cotton, can be spread large distances in soil, and re-wetting events will cause organisms to redistribute particularly within the surface soil.

Chemistry

Soil chemistry can influence the rhizosphere organisms directly, or indirectly by modifying root growth and exudates (see Rengel and Marschner 2005; Nelson and Mele 2006, this issue). Australian soils are generally low in available phosphorus and added phosphorus is quickly bound or 'fixed' to soil surfaces, or is bound within organic matter. Soils can be too acidic or alkaline, or have high salt concentrations or toxic levels of elements such as Al, Mn, and B. Many herbicides applied to weeds either drip from leaves into surface soil, or are incorporated into surface soil. Some, such as sulfonylurea, can increase damage to roots by *Rhizontonia* (Smiley and Wilkins 1992), possibly by inhibiting root extension (Wheal *et al*. 1998).

Fig. 2. (*a*, *b*) Bar = 2 mm. Apical regions of seminal roots of wheat cv. Janz, harvested at one-leaf from a paddock in south-eastern Australia. Apex in (*a*) has a long zone of elongating cells (e) behind the root meristem (m) where cells are dividing, indicating that it is extending through soil with little structural impedance. Root hairs (h) are developing and extending into soil behind the elongating zone. The extreme tip is surrounded by a root cap (c), which produces cells and mucilage that binds soil as the tip advances. Apex in (*b*) has been impeded by the soil, and is distorted (arrowhead). Roots hairs and bound soils are immediately behind the meristem (m) and there is no visible elongating zone, suggesting that it is growing very slowly. *Pseudomonas* and other bacteria accumulate in higher numbers behind the tips of roots such as (*b*). (*c*) Flank of a root tip of wheat (arrowheads indicate the edge of the root) such as that shown in (*b*), harvested from a paddock in southeastern NSW. Root has been exposed to DNA probes with fluorescent dyes: EUB338-Cy5.5 (yellow) targetted to hybridise to all bacteria and PSE227-Cy5 (blue) targetted to *Pseudomonas* bacteria, using FISH (fluorescent *in situ* hybridisation; see Watt *et al*. 2006*a*), and viewed with a confocal scanning laser microscope. The root tip has single-celled bacteria that include *Pseudomonas* (approx. 10% of bacteria) and filamentous bacteria (approx. 4% of bacteria; inset) bound to the root and its associated soil (s). Bar for both images = 20 µm. (*d*) Roots clumped in a pore (p) approximately 0.3 m from the soil surface. Wheat was sown after 3 years of lucerne pasture. Some of the roots emerging from the pore are wheat axes with short branch roots (arrow); others may be remnant from lucerne or weeds (possible remnant indicated by arrowhead). Image taken in the field, after roots were exposed by digging pits 2 m deep around the crop, using a tractor. Bar = 10 mm. (*e*) Surface of a wheat root (w) harvested from a pore such as that shown in (*d*). Root remnants (rr) from previous crops are intimately associated with the wheat root hairs (arrows). The remnants have abundant bacteria, especially filamentous bacteria, that also colonise the wheat root hairs (see f, g). Bar = 100 µm. (*f*, *g*) Wheat root (w) processed using FISH and observed with a confocal microscope as in (*c*) to show the extensive bacteria on the remnant roots (rr), especially filamentous bacteria (yellow filaments). These filaments and other bacteria are also seen on wheat root hairs (h) close to the remnants. (*f*) is the brightfield view of (*g*). Bar = 60 µm. (*h*) Core of soil taken from below a wheat plant at anthesis, pushed onto a cradle, and broken 0.6–0.7 m from the soil surface to reveal mainly wheat roots (arrow), and some remnant roots (arrowhead) that had grown within a plane of weakness in the soil. Bar = 1 cm. (*i*) Wheat root from 1.5 m below the soil surface, harvested as in (*h*), and processed with the fluorescent dye, DAPI (4,6 diamidine-phenyl indole; see Watt *et al*. 2003), to visualise all bacteria (arrows to bright spots) with a microscope and UV light. The root surface has many bacteria at the deep soil, and a hypha (double arrowhead).

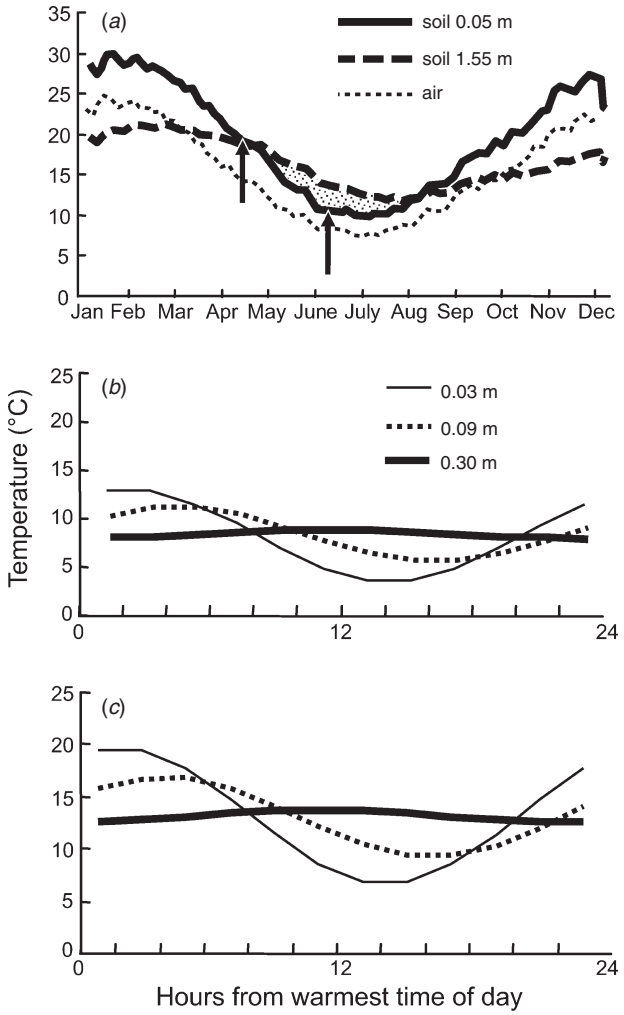

Fig. 3. (*a*) Mean temperature of the air between 1990 and 2004 at Harden, south-eastern NSW, through the year taken from weather station data. Soil temperatures at the surface and at depth were estimated using the APSIM model. Running means over 7 days are shown. The soil at 0.05 m oscillates approximately 20°C, and approximately 9°C at depth over the year. Deeper soil is warmer than surface soil during the winter (shaded area), and hence roots grow into warmer temperatures during young crop growth. Roots of crops sown in April encounter temperatures 10°C warmer than in June (arrows). Soil is always warmer than the air. Hourly soil temperatures over a day in (*b*) south-eastern NSW (Harden) and (*c*) northern QL (Darling Downs, Dalby Airport) in mid-August. Values at different depths were calculated from the long-term average maximum and minimum air temperatures using equations developed by West (1952).

The surface soil can be readily ameliorated, with lime or gypsum to alleviate acidity or sodicity, or with fertilisers as required. Subsoils are much less readily affected by management, and toxic elements or physicochemical hostilities, such as severe sodicity, can severely limit root depth and thence crop production. The behaviour of subsoil roots and their rhizospheres, particularly perennial rhizospheres in biopores that are repeatedly colonised by successive generations of roots and that contain many remnant roots, may hold the key to improving how root systems of current crops can make better use of subsoils.

Table 2. Rates of descent of root systems at 2 temperatures within upper and deeper depths of a pre-packed soil column

From Cohen and Tadmor (1969). Plants grown in sandy loam soil packed to 1.35 g/cm^3 bulk density in columns, in growth chambers. Radioisotopes were placed at 0.12 and 0.22 m from the soil surface, and shoots analysed for the isotopes by harvesting a few plants per column over time. Values in parentheses are factor increase between 10 and 20°C

Depth:	Rate of root system descent (cm/day) 0.02–0.12 m		0.12–0.22 m	
	10°C	20°C	10°C	20°C
Triticum aestivum (wheat)	2.5	7 (2.8)	1.5	3.2 (2.1)
Hordeum vulgare (barley)	3.5	7 (2)	2.2	3.2 (1.4)
Avena sterilis (wild oats)	0.9	3.2 (3.5)	0.9	1.5 (1.6)
Phalaris tuberosa (Harding grass)	0.3	1.1 (3.6)	n.d.	0.6
Vicia dasycarpa (vetch)	1	3 (3)	0.4	1.5 (3.75)
Medicago truncatula (medic)	0.5	1.5 (3)	n.d.	0.8
Agropyron elongatum (tall wheat grass)	0.5	1.1 (2.2)	n.d.	1.1

n.d., Not determined because roots did not reach the deeper level within the duration of the experiment.

Agronomic effects linked to rhizosphere processes

In this section we present three case studies in which significant agronomic effects can be linked to rhizosphere processes. In each, understanding the interactions of roots and organisms in field-relevant rhizospheres (as discussed in the previous section) was necessary to understand and benefit from the different agronomic interventions under investigation. Other examples of such agronomic effects related to rhizosphere processes include optimising specific rhizobia to legume combinations (Brockwell *et al*. 1995), and management to encourage proliferation of specific disease-suppressive organisms in the soil (see Barnett *et al.* 2006, this issue).

Case study 1: productivity of direct-drilled wheat in SE Australia

Conservation cropping systems involving direct-drilling of crops into uncultivated seedbeds have been developing for over 30 years in Australia, and systems have been tuned to specific regions (Steed *et al*. 1993). The level of adoption of direct-drilling has varied significantly within different regions of Australia, from an estimated 98% of crops direct-drilled in the sand-plains of Western Australia to only 13% in central NSW (Connell and Hooper 2002). In southern Australia, particularly in higher rainfall areas, adoption has been slow and the benefits to crop yields flowing from

seemingly improved soil conditions harder to demonstrate (Kirkegaard 1995). There are several contributing reasons: stubble loads are generally higher, erosion risks are lower, regular pasture phases maintain soil organic matter, the incidence of soil- and stubble-borne disease is high, and, in many winter-dominant rainfall areas, the conservation of water is less critical for crop yield.

From the outset, a consistent problem with direct-drilled wheat in southern Australia was the reduced early vigour of crops compared with those sown into cultivated soil (Kirkegaard *et al*. 1995; Simpfendorfer *et al.* 2002). In a survey of glasshouse and field studies worldwide, Lekberg and Koide (2005) also found evidence for constrained growth (14% shoot biomass reduction) in direct-drilled crops compared with those in disturbed soil. Causes for this constrained growth include changes in temperature and water content of the surface soil, reduced nutrient availability and/or uptake, increased soil strength and reduced root growth, increased incidence of foliar and root disease, and increases in inhibitory microorganisms and phytotoxins. The surprising results of Chan *et al*. (1987) and later Kirkegaard *et al*. (1995), showing that soil fumigation could overcome the early growth reductions, pointed to the role of soil biological constraints.

In a subsequent investigation at 39 farm sites over 3 years in southern NSW, Simpfendorfer *et al*. (2001, 2002) demonstrated that the problem was widespread (62% of sites), was not related to any of the major soil-borne cereal disease organisms, or to general changes in soil biology, but was strongly related to the inhibitory activity of *Pseudomonas* isolated from the rhizosphere of wheat seedlings at each site. The most likely mechanism for this effect was recently elucidated by Watt *et al*. (2003, 2005) who studied the architecture, distribution, morphology, and associated soil biology of intact field-grown roots of direct-drilled wheat at a long-term tillage experiment at Harden in southern NSW, where reduced early growth had persisted for many years. The studies showed a higher proportion of contorted, slow-growing root tips constrained by the harder direct-drilled soil, and an associated build-up of *Pseudomonas* on the slow-growing root tips. The *Pseudomonas* preferentially built up in the zone around the root tip, whereas the general rhizosphere bacterial population did not.

Thus an interaction between the intact field structure and a specific component of the soil biology was generating a pattern of rhizosphere colonisation that was associated with inhibited wheat growth in direct-drilled soil. This finding could explain why management strategies such as early sowing into warmer soils and cultivation below the seed, both of which increase seedling root growth rates, reduced the effect of direct-drilling on early growth. It also provided opportunities to explore other management and/or genetic options to increase the rate of root growth to avoid the problem (Watt *et al*. 2005). This work provides a good example of the importance of examining the intact soil/root system in the field when trying to unravel puzzling plant growth responses, as well as the importance of interactions between the soil biology, the soil structure, and the patterns of root growth, in determining those responses.

During the course of this work, the long-term field site at Harden attracted other research on the effect of conservation cropping on soil biology, mostly concerned with effects on the populations of particular classes of organisms. The widely promoted improvements in soil biology expected under direct-drill systems were also evident at the Harden site where increases in soil organic matter, microbial biomass, populations of earthworms, nematode and faunal diversity, as well as disease suppression were all evident on direct-drill/stubble-retained treatments compared with late-burn/single-tine-cultivation treatment (Table 3). In spite of these general 'improvements', the growth and yield of wheat throughout the 15-year period have been lower on the direct-drill/stubble-retained treatment compared with the most commonly used management system in the region, comprising a late-burn/single cultivation prior to sowing (Table 3). In addition, more residual subsoil water and mineral N remained in the soil following harvest of direct-drill/ stubble-retained crops, representing an increased risk of deep drainage and N leaching under the conservation cropping system. Thus, in these high-rainfall, mixed farming systems in south-eastern Australia, the promotion of direct-drill/stubble-retained systems on the basis of 'improvements' in aspects of the soil biology, and promoted as soil 'health' or 'quality' (see also Letey *et al*. 2003), may overlook the associated production constraints and sustainability issues such as acidification and salinisation.

Case study 2: the role of arbuscular mycorrhizal fungi (AMF) in wheat production

AMF are obligate symbionts that colonise the roots of most crop plants, taking up nutrients such as P and Zn in return for assimilates from the host. They have also been credited with improving soil structure through their external hyphal structures and the production of the polysaccharide glomalin (Wright and Anderson 2000), increasing water availability, and suppressing disease (Graham 2001). As a major component of the below-ground ecosystem, their potential importance in crop production has been studied intensively. However, their effects on productivity of agricultural systems have been difficult to assess and contradictory (Ryan and Graham 2002; Lekberg and Koide 2005).

The clearest example of AMF benefits to crop growth in the field in Australia is on Vertisols in the northern wheatbelt, where crops grown after 12–18 months of bare fallow grew poorly owing to P and Zn deficiencies associated with low levels of AMF, a condition known as 'long-fallow disorder' (Thompson 1987). Such a problem can be managed in the field either by avoiding sequences of fallow or non-

Table 3. Effects of conservation cropping on soil and crop parameters at the long-term field site at Harden, NSW
Conservation cropping (stubble retain/direct drill) is compared with orginal district practice (late stubble burn/tine cultivate)

Parameters	Effect of conservation cropping	Reference
	Soil biology	
Organic C/N	Increased	Kirkegaard *et al*. (2001)
Microbial biomass	Increased	Gupta (1994)
Earthworms	Increased	Doube *et al*. (1994)
Nematodes	Increased abundance/diversity	Hodda *et al*. (1997)
Soil fauna	Increased	Longstaff *et al*. (1999)
	Wheat rhizosphere biology	
Total fungi	No effect	Simpfendorfer *et al*. (2002)
Total bacteria	No effect	Simpfendorfer *et al*. (2002)
Total pseudomonads	No effect	Simpfendorfer *et al*. (2002)
Inhibitory pseudomonads	Increase	Simpfendorfer *et al*. (2002)
Root tip pseudomonads	Increase	Watt *et al*. (2003)
Root pathogens	No effect	Simpfendorfer *et al*. (2002)
Disease suppression	Increase	Pankhurst *et al*. (2002)
	Disease incidence	
Rhizoctonia patches	Increase in medium term	Kirkegaard *et al*. (1994)
Yellow leaf spot	Increase	Kirkegaard *et al*. (2001)
	Crop performance (wheat)	
Vegetative growth	Reduced by 30%	Kirkegaard *et al*. (2001)
Yield	Reduced by 11%	Kirkegaard *et al*. (2001)
	Systems issues	
Aggregate stability	Increased	J. Kirkegaard (unpublished)
Infiltration rates	No change	J. Kirkegaard (unpublished)
Residual N and water	Increased	Kirkegaard *et al*. (2001)
Deep drainage	Increased	Kirkegaard *et al*. (2001)

host followed by AMF-dependent crops, or by ensuring that P and Zn nutrition of the following crop is adequate. The latter is sometimes problematic on these soils, which are prone to extended periods of surface drying, making P and Zn fertilisers unavailable. Observations that less dependent crops such as wheat could also be affected when grown after non-host crops such as canola raised concerns regarding the general effect of canola on AMF and wheat productivity, not only in the northern wheatbelt (Thompson *et al*. 2001), but also elsewhere in Australia, because canola and another non-host, lupin, were the most widely grown broadleaf break crops rotated with wheat in the southern and western wheatbelt.

A comprehensive study of the effect of AMF on wheat productivity in south-eastern Australia on both alkaline Vertosols similar to those in the northern wheatbelt and on acidic Kandosols was conducted by Ryan and colleagues (Ryan *et al*. 2002; Ryan and Angus 2003). They manipulated levels of AMF in commercial fields and in previously uncropped soils by using combinations of different pre-crops that varied in host status, P fertiliser application, and cultivation, to generate AMF root colonisation, varying from 5 to 70%, in subsequent wheat and pea crops. They also monitored a range of other important agronomic parameters including soil water, mineral N, and soil-borne root pathogens to enable clear interpretation of the results under commercial field conditions. They showed that high AMF colonisation in wheat and field pea did not increase nutrient uptake, biomass, or yield in autumn-sown crops in spite of a strong P limitation on crop growth and yield. The authors concluded that high colonisation by AMF is unimportant for the productivity of wheat or field pea grown on these soils, which occupy large areas of cropland in temperate south-eastern Australia. In some experiments, higher AMF colonisation led to greater uptake of Zn and P after anthesis and higher grain concentrations, suggesting a great activity of AMF late in the season. The authors hypothesised that for these autumn-sown crops, cool soil prior to spring reduced nutrient uptake by AMF, and that AMF was likely to be parasitic then. This hypothesis is supported by the lower levels of water-soluble carbohydrates and reduced growth of seedlings as AMF colonisation of the roots increased (Ryan *et al*. 2005). AMF commonly require up to 20% of total fixed host photosynthate to support their colonisation of roots, and parasitism in the absence of nutritional benefits has been documented for other crops (Graham 2000). Rather than reducing nutrient uptake and productivity, lower levels of AMF colonisation may partly explain the superior growth of wheat following

non-host crops such as canola in south-eastern Australia, due to reduced drain on C from the seedling roots. On highly calcareous P-fixing soils elsewhere in southern Australia (such as upper Eyre Peninsula), recent studies showed that fumigation could significantly reduce wheat growth in the absence of applied P, suggesting a role for AMF and other P-solubilising microorganisms in those areas, although this problem is readily addressed in wheat when commercial rates of P are applied (D. K. Roget, CSIRO Adelaide, pers. comm.). As for the northern Australian case, the growth of more highly AMF-dependent pasture species such as lucerne or medic could not be restored with fertiliser application following fumigation, and for successful establishment of these species, AMF management is likely to be more critical.

These studies highlight the need for careful studies in the field to quantify the importance of AMF for different crops within specific farming systems.

Case study 3: Brassica *break crops and biofumigation*

Substantial productivity improvements in Australian wheat crops in the last decade were underpinned by controlling root diseases using broadleaf break crops such as canola (*Brassica napus*) and lupin (*Lupinus angustifolia*) grown in sequence with cereals (Angus 2001). Kirkegaard *et al*. (2004) reviewed yield responses of wheat to preceding break crops and the mechanisms responsible. They concluded that the average yield improvement of 20% in wheat was remarkably consistent across broad regions and time scales and that much is known about the mechanisms responsible such as disease control, improved nutrition, and water supply. However, there remained inexplicable 'rotation' effects apparently associated with poorly understood or inadequately defined factors, particularly soil biology and soil structure.

An interesting case study in this regard is the effect of canola on wheat crops in south-eastern Australia, where wheat grew better following *Brassica* break crops than when following other broadleaf break crops in the early 1990s. Angus *et al*. (1991) and Kirkegaard *et al*. (1994) explored possible causes, and could not attribute the effect to the non-hosting of root disease because all break crops were non-hosts, or to nitrogen nutrition. One possibility was that *Brassica* crops were improving the soil structure, both in the surface layers as a result of their extensive fine roots (Chan and Heenan 1996) and in the subsoil as a result of biopores created by their deep taproots, which were used by subsequent wheat crops to penetrate the soil. However, Cresswell and Kirkegaard (1995) subsequently found no evidence that canola could improve subsoil structure, and although Schönhammer and Fischbeck (1987) had previously found some evidence for improved soil structure following canola, such effects would be transient under the conventional cultivation regimes used in Australian canola production at the time.

Another hypothesis was that allelochemicals unique to brassicas, principally isothiocyanates (ITCs), may actively suppress disease organisms in a process termed 'biofumigation' (Kirkegaard *et al*. 1993; Angus *et al*. 1994). In this process, the ITCs were thought to be released during canola root growth or decomposition, reducing the levels of disease inoculum to infect subsequent wheat crops. Subsequent laboratory and pot studies demonstrated that cereal pathogens such as take-all (*Gaeumannomyces graminmis*, *Ggt*) were highly sensitive to the ITCs released by canola roots, whether in a pure form applied in Petri dish agar (Sarwar *et al*. 1998; Smith and Kirkegaard 2002), or when canola root tissues were added to soil at rates likely to be present in the field (Smith *et al*. 1999). A subsequent series of field experiments showed that *Ggt* inoculum fell to lower levels under canola crops than under linseed crops during the period from flowering to maturity (Kirkegaard *et al*. 2000). This coincided with a fall in concentration of the ITC-precursor glucosinolates (GSLs) in the canola taproots.

These results supported in part the original 'biofumigation' hypothesis: GSLs contained predominately in the canola tap roots were released and hydrolysed when roots decomposed late in the season and reduced the levels of *Ggt* inoculum compared with non-*Brassica* break crops. However the differences in *Ggt* inoculum measured at the time of canola harvest did not always persist during the subsequent 5-month summer fallow prior to the following wheat crop, as *Ggt* inoculum declines whenever there is no host present and soil moisture facilitates decomposition. As a result, the effects of the *Ggt* suppression during the canola year on the disease development and yield in subsequent wheat crops were limited. This was confirmed by Smith *et al*. (2004) who failed to detect any evidence that brassicas influenced the levels of *Ggt* or other rhizosphere organisms on the roots of subsequent wheat crops differently from other break crops, raising further doubt about 'biofumigation' as it was originally conceived. Thus, despite reports of significant ITC-induced changes in the rhizosphere bacteria of canola (Rumberger and Marschner 2003), it appeared that such effects did not necessarily persist to influence the levels of disease in a subsequent season.

Recent studies of ITC concentrations in the soil around canola roots and the conditions necessary for their release, together with broader consideration of the overall effects of canola on the soil biology in crop rotations, indicate that the original biofumigation hypothesis was simplistic. GSLs and the myrosinase enzyme necessary for the hydrolysis to form ITCs are physically separated in intact tissues, so that significant tissue disruption is required for significant ITC release. Rumberger and Marschner (2003) measured mean ITC concentrations of 0.5 nmol/g and maximum concentrations of 1.8 nmol/g in the rhizosphere of canola grown in glasshouse rhizotrons, whereas only traces were found in the bulk soil (Table 4). ITC added to soil was

Table 4. The concentration of isothiocyanates (ITCs) measured in soil in canola rhizospheres, following incorporation of *Brassica* tissues after different degrees of tissue disruption, or after commercial ITC fumigation

Situation	ITC conc. (nmol/g soil)	Reference
Canola rhizospheres glasshouse (disturbed)	0–1.8 (mean 0.5)	Rumberger and Marschner (2003)
Canola rhizospheres in field (root-proof bags)	0–1.0 (mean 0.06)	Smith *et al*. (unpublished)
Canola green manure (after rotary incorporation)	0.8	Morra and Kirkegaard (2002)
Canola green manure (mulch/incorporate/irrigate)	20	Matthiessen *et al*. (2004)
Mustard – leaf tissue (freeze/thaw)	100	Morra and Kirkegaard (2002)
Commercial ITC fumigation	300	Matthiessen *et al*. (2004)

degraded by microorganisms within 96 h. The concentrations of ITC measured periodically in soil, within root-proof pouches buried adjacent to canola plants growing in the field, also did not exceed 1 nmol/g, and were, more often than not, undetectable (B. Smith and J. A. Kirkegaard, unpublished). Morra and Kirkegaard (2002) showed that less than 1% of the potential ITC was released in the field (0.8 nmol/g soil) following rotary cultivation of flowering canola crops into the soil, whereas full tissue maceration and irrigation could increase this to 20 nmol/g soil (Matthiessen *et al*. 2004). Freezing leaf tissue released around 30% of available ITCs into soil upon thawing (100 nmol/g soil) which is approaching the levels of ITCs detected following commercial fumigation (300 nmol/g soil) reported by Matthiessen *et al*. (2004) (Table 4). These results show that the levels of ITC released in soil from canola roots in broad-acre production are likely to be too low for biofumigation, except perhaps for the most sensitive of soil organisms such as *Ggt*.

Although biofumigation to directly influence pathogenic fungi such as *Ggt* seems unlikely due to the low ITC concentrations in the rhizosphere of dryland canola, these or other compounds specific to *Brassica* rhizospheres can influence the rhizosphere biology (Rumberger and Marschner 2003), and in some cases this can significantly influence following cereal crops. For example, Kirkegaard *et al*. (2004) showed that *Brassica* break-crops led to higher levels of the *Trichoderma* spp. isolated from the crowns of following wheat crops than after chickpea or cereal crops. *Trichoderma* are known antagonists of cereal disease such as crown rot (*Fusarium pseudograminiearum*) and have been shown to be highly tolerant of ITCs *in vitro* (Smith and Kirkegaard 2002). Further evidence that *Brassica* break crops could significantly affect soil biology was that different amounts of mineral nitrogen accumulated in the summer fallow following brassicas than following legumes (Kirkegaard *et al*. 1999*a*), an observation that could not be explained by the amount, nitrogen content, or carbon : nitrogen ratio of the crop residues. What caused this effect is uncertain, but populations of organisms associated with nitrogen cycling such as free-living nitrogen-fixing bacteria, *Azospirillum* spp., and ammonium-oxidising bacteria were generally *lower* following canola, whereas total bacterial populations did not differ. Further studies (Ryan *et al*. 2006, this issue) have shown that the effects that accelerate mineralisation are transitory under laboratory conditions, but in the field they are strongly influenced by the growth of subsequent wheat crops.

It is now clear that the rotational benefits of *Brassica* break crops can derive from many effects on soil biology in addition to the reduction in hosting of cereal pathogens. Although the specific effects of ITCs on rhizosphere biology cannot be ruled out, most of these effects appear to be general changes in rhizosphere organisms, rather than the direct killing of disease inoculum by ITCs.

Future directions

Research

Field research

Research in the field is essential to link rhizosphere biology and crop productivity. The role of the agronomist as integrator is crucial. Quantifying rhizosphere biology in the field may involve direct harvesting and analysis of crop roots and their rhizospheres, or the use of fumigants and other chemicals toxic to specific organisms. With the former, careful consideration is needed as to when and where rhizosphere organisms can be expected on different root types in the profile, based on a good estimation of the dynamics among soil structure, temperature, moisture, and chemistry (Watt *et al*. 2006*b*). With fumigants, caution is needed regarding confounding interactions with nutrients released from killed cells. Such treatments are gross disturbances of the soil organisms at best, rather than complete sterilisation, but nevertheless remain our best method for assessing pervasive roles for soil organisms in cropping systems.

New methodologies

Molecular methodologies have dominated the study of rhizosphere organisms in the past 20 years (reviewed in Prosser 2002). Nucleic acids can be extracted from soil or isolated organisms, sequenced, and positioned on phylogenic trees to identify previously uncultured organisms and their diversity (Marschner *et al*. 2001; Johnson *et al*. 2003). Uncultured organisms that divide in response to substrate can be identified (Borneman 1999). Molecular methods are

combined with more traditional techniques such as BIOLOG to assess which substrates are used by isolated organisms, and Janssen *et al*. (2002) have used novel culturing methods with such studies to culture up to 19% of microscopically counted cells from soil (many times more than previously possible) to characterise the physiology of the isolates.

Oligonucleotide probes can be designed to quantify (e.g. with Real Time PCR) rDNA or rRNA in extracted samples to, for example, predict root disease on farmers' crops. Combined with knowledge of summer rain and breakdown of leaf substrates that host the disease, these have been particularly successful in predicting the dynamics of Take-all disease of wheat in South Australia (D. K. Roget, unpublished). Such analyses will continue to be valuable to combine with yield mapping and knowledge of soil type and other paddock-scale attributes to help manage spatial variability of yield.

Oligonucleotide probes are conjugated to fluorochromes to bind to rRNA of organisms on roots for direct visualisation (fluorescence *in situ* hybridisation, FISH) (Amann *et al*. 1995). FISH is powerful for showing where bacteria are on roots in relation to other features identified in the same microscopic field of view, particularly when combined with the 3-dimensional capabilities of laser confocal microscopy. However, the field of view is small compared with even a single root (<1% of the rhizosphere of a 1-cm piece of root), and FISH cannot be used to detect gross treatment differences at the paddock scale. Probe number is limited by interference from soil particles that emit in the same range as fluorochromes (see Bouvier and Del Giorgio 2003 for comprehensive review of limitations of FISH), and the organisms observed are those left behind after sample preparation. FISH was recently used to quantify rhizosphere bacteria on wheat roots grown in the field (Watt *et al*. 2006*a*). *Pseudomonas* constituted 10% of the total labelled bacteria, and was present in numbers 10–100 times *less* than evident in controlled environment studies reported in the literature. However, root caps were heavily colonised by bacteria, and contact points with remnant roots had more filamentous bacteria than other regions.

Reporter genes are inserted into bacteria or fungi to express ice-nucleating or fluorescing proteins (generally *lux* or the green fluorescing protein, *gfp*). These may express continuously (Bloemberg *et al*. 2000), or in response to a chemical process in those organisms (with an inducible promoter), which may be related to a rhizosphere exudate or signal (Jaeger *et al*. 1999; Steidle *et al*. 2001). The transformed bacteria are generally viewed *in situ*, and sometimes combined with FISH to identify associated organisms. Such 'biosensors' can help identify local chemistry in the rhizosphere; however, as with FISH, samples must be very well defined because only a small area of the rhizosphere is viewed at any one time under a microscope. Larger areas can be seen with a CCD camera at the cm scale, such as in the study of carbon efflux from barley root systems (Darwent *et al*. 2003), or by extracting cells and combining with flow cytometry. Sample preparation and what compounds remain in the rhizosphere (volatiles, water-soluble ones) over what time frame for biosensors to express are also important, as is soil autofluorescence, which will restrict the types of biosensors.

Novel imaging techniques, adapted largely from medical and earth sciences, combined with *in situ* organism interactions, will give insights into rhizosphere structure and processes across a broad range of organisms. These include cryo-analytical scanning electron microscopy to localise phosphorus concentrations to arbuscular mycorrhizas grown in soil (Ryan *et al*. 2003) and necrotrophic fungi such as *Rhizoctonia* within rotting roots (Refshauge *et al*. 2006), and synchrotron-based methods to image and quantify mineral-organic complexes on and within roots (Hansel *et al*. 2001). Computed tomography (CT) offers opportunities for non-invasive imaging of root–water–organism interactions (Grose *et al*. 1996; Johnson *et al*. 2004). Improvements in resolution and imaging software will allow studies in larger volumes of soil, and distinction between water, organic material, and solids in intact field soil. These exciting technologies need to be combined with existing, long-standing techniques to relate to processes at different scales.

Consistent units for rhizosphere processes

Soil bound to roots after excavation from pots or the field is used as the 'rhizosphere' in many studies. However, this depends on root hair length (e.g. 1 mm for barley), root and organism mucilages, and water (drier soil increasing hair length and soil adhesion, Watt *et al*. 1993, 1994). Further, bacteria such as *Cytophaga* were more associated with soil tightly bound to barley roots, compared with *Pseudomonas* which were associated with the loosely bound soil (Olsson and Persson 1999). Using adhered soil will overestimate or underestimate different rhizosphere organisms and processes (Hinsinger *et al*. 2005). Different studies are thus analysing different fractions of the rhizosphere biology that depend on the spatial and temporal definition of the rhizosphere, adhesion of organisms to roots and soil, and methods of extraction.

The behaviour of rhizosphere organisms is rarely followed through time. There is a lack of consistent units to express dynamic processes. This is a major problem for comparing studies since expression per length, weight, or volume of root or soil gives different numbers (Duineveld and van Veen 1999). This lack of consistency means that we often have little idea if some process in the rhizosphere is happening quickly or slowly compared with something else, which makes it difficult to connect a given rhizosphere process to agronomic practices and yield. Expression of roots and organisms in units of distance and time (rates) helps to reveal how the rhizosphere develops in different cropping

conditions, such as direct-drilling or biopores in the subsoil, and how it thus can be managed with agronomy or breeding (Watt *et al*. 2006*b*).

Prospects to harness rhizosphere processes in novel ways to improve crop performance

We have emphasised the central role of roots in regulating soil biology. Here we suggest 4 ways that roots could be used to help manage soil biology. They all use roots of different genotypes to improve plant growth. The greatest gains will be through targetting specific traits of different plants to specific farming systems. For example, a wheat genotype developed for vigorous leaf growth was recently found to be less affected by soil organisms in direct-drilled soil compared with a conventional cultivar, Janz (Watt *et al*. 2005). Thus, vigorous genotypes may present a new opportunity for increasing productivity in conservation farming.

Manipulating roots and exudates of the current crops

Genotypes could be selected with roots and exudates to modify the rhizosphere biology to benefit the current crop (O'Connell *et al*. 1996; Rengel and Marschner 2005). For example, genotypes may vary in the extent that their rhizospheres overlap because their root axes respond differently to gravity, resulting in different root architectures (Ge *et al*. 2000). The extent of rhizosphere overlap would create differences in net concentrations of exudates around roots and thus the numbers and diversity of some microorganisms. Differences in root-hair length may change the size of the rhizosphere and extent of close contact between soil organisms and the root, and 2-fold variation in root-hair length and density has been identified in barley (Gahoonia *et al*. 1997). Neal *et al*. (1973) and Miller *et al*. (1990) reported differences in bacterial populations between 2 wheat genotypes that differed in one chromosome. Azcón and Ocampo (1981) found wide variation in wheat cultivars for VA infection, which was not related to nutrition, and possibly related to carbon efflux from roots. More recently, the more vigorous wheat line, V18, was less stimulated by fumigation compared with the conventional cultivar, Janz, suggesting that its roots either did not host organisms detrimental to growth, or that it was less affected by such organisms (Watt *et al*. 2005). V18 had fewer *Pseudomonas* on its root tips from the field compared with Janz (unpublished data). This suggests that there is genotypic variation in the amount or type of exudates from the root tips. Gupta *et al*. (2004) showed 3-fold variation in the number of copiotrophic bacteria ('fast-growing') on the roots of wheat cultivars grown after a preceding cereal crop, and postulated that Trident, a wheat cultivar that grows well after previous wheat crops, induces smaller populations of copiotrophic bacteria and larger populations of oligotrophic bacteria in its rhizosphere, due to either the amount or composition of exudates. It appears that larger populations of copiotrophic bacteria (e.g. *Pseudomonas*) relative to oligotrophic bacteria may be detrimental to the performance of some wheat varieties, and that pre-crop species and genotypes can influence this ratio.

Exploiting remnant roots of cereals

Based on the evidence in the previous section, it follows that genotypes could be selected with roots and exudates that, as remnants, host organisms that benefit the subsequent crop. This 'rotation' has been extensively exploited in cereal farming systems using legume and oilseed break crops (Table 1); however, attention could now focus on the variation in the rhizosphere effects of remnant cereal roots. Mazzola *et al*. (2004) showed that one wheat cultivar out of 6 stimulated the presence of DAPG-producing, disease-suppressive *Pseudomonas* strains in soil. Sowing these cultivars may speed up natural suppressiveness in paddocks. The chances for success in using crop or remnant roots to improve soil biology will depend on agronomic history and soil type. It may be that soils low in organic matter and remnant roots, such as sands or newly cropped soil, will make for distinct rhizospheres, whereas soil with high organic matter and remnant roots may swamp the developing rhizospheres with pre-existing populations of organisms on the background organic materials (Garbeva *et al*. 2004).

Using specific genotypes for delivering inoculants

Substantial research has gone into developing strategies for inoculating seeds and soil with organisms that can stimulate crop growth in the laboratory, but few inoculants are successful in the field (Stewart 2001). A better understanding of what parts of root systems inoculants come from could help improve how inoculants perform in the field. An important trait is the ability to keep pace with the growing root tip of a main axis. Simons *et al*. (1996) selected inoculants from the tips of roots that had been growing the longest, and found that a specific root tip exudate was one factor critical to some isolates keeping pace with the root tip. McCully (2001) proposed that endophytic inoculants are likely to be living in decaying cortices of roots. Such spaces could be exploited as niches to encourage inoculants to proliferate. If an inoculant applied with the seed proliferates in decaying cortical cells, genotypes with more and faster cortical decay at the root base may be more likely to support the inoculant. Residues from previous crops can be used to host inoculants (Bowen 1980) such as beneficial actinomycetes, or suppressive organisms such as those reported in Barnett *et al*. (2006, this issue).

Using roots to develop good soil structure

Roots change the soil structure by growing between aggregates and reshaping the spaces within soil. They are a powerful management tool. Worldwide, roots of lucerne are used to create 'biopores' in deeper soil

layers that a subsequent crop can use (Cresswell and Kirkegaard 1995; Davies and Peoples 2003). Root systems can also be used to improve soil structure in the surface soil. Over time, agricultural soils typically harden. Such 'coalescence', particularly obvious on irrigated soils (Cockroft and Olsson 2000), can be reversed with ryegrass roots, accompanied by appropriate tillage and by gentle irrigation (B. Cockcroft, pers. comm.). This reversal appears to be driven primarily by how populous ryegrass roots and hairs are, and by accompanying mucilages that bind soil aggregates.

Conclusions

Major leaps in the productivity of agricultural systems rarely arise from interventions related to single factors, but rather from synergistic interactions among many interventions working together. This is most famously demonstrated by the English agricultural revolution in the 1700s in which the synergistic interactions among the individual components of the Norfolk system — use of marl and clay, rotation of crops, the culture of turnips hand-hoed, and the culture of clover and rye — most of which had been promoted individually since ancient times, made it such an effective agent of improvement (Evans 1998). More recent examples of such effective interactions in Australian agriculture include the 'pasture improvement revolution', involving adapted legume species, inoculation with effective rhizobia, application of P and in particular molybdenum so vital for the effective activity of rhizobia (Williams and Andrew 1970), and the more recent doubling of average wheat yields in south-eastern Australia underpinned by root-disease control using break crops such as canola, and the consequent responses of semi-dwarf varieties to N fertiliser applications (Angus 2001).

These examples serve to illustrate the need to carefully consider any planned manipulation of soil biology to improve crop production in the context of both the future farming systems in which we are expecting such interventions to be effective, and the actual root and rhizosphere environment in which we expect them to function. The current trend in dryland farming systems towards no-till farming with controlled traffic or precision guidance systems, together with other economic imperatives to increase the scale and efficiency of operations, are likely to continue. Aspects of these systems relevant to rhizosphere biology include the preservation of intact soil structure and the increased longevity of root residues from season to season, as well as a capacity to more precisely deliver seed, fertilizer, and other products in the soil.

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