Interaction between soil microbiome and nematode community regulates crop growth

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Abstract

Nematode communities represent the most diverse and abundant multicellular fauna in the soil ecosystem, and the multi-feeding channels of nematodes provide an important linkage within the soil food web, as well as between soil and plant interactions. However, given the complex connection in the soil microbiome and the diversity of soil nematodes, their contribution, relative to other soil fauna, on enhancing plant growth and development rather than hindering growth remains largely unknown in soil ecosystem. In this experiment, we initially separated soil nematode communities from the remaining microbial communities (including bacteria and fungi), and then re-introduced either or both communities to a sterilised soil. Non-sterilised soil was also included. The aim of the study was to investigate the role of the soil nematode community and indigenous soil microbial community on the growth of wheat and canola, using three different soil types from South Australia and Victoria. Plant growth was monitored using high-throughput imaging over six weeks; soil was tested at the end of the experiment, including sequencing for microbial community and qPCR for nematode community. Plant growth was more strongly influenced by sterilisation, irrespective of crop type, rather than by the addition of either nematode or microbial communities. Among all treatments with sterilised soil, the addition of either nematodes or microbial communities altered the growth rate for both canola and wheat, with the effect being stronger for canola. These findings provide a new insight into the role of different components of the soil food web on crop production.

Keywords

Bacteria; Canola; Fungi; Nematode communities; Soil food web; Soil microbial community; Wheat

Introduction

Soil ecosystems comprise communities of extreme complexity and diversity, with millions of species and individual organisms found within a handful of soil, ranging from microscopic bacteria and fungi to soil meso and macro fauna[1]. At the fundamental trophic level, soil microbial communities are the key component determining important soil processes, such as the supply of nutrients and water to plants, and establishment of symbiotic/parasitic relationship with plants[2]. Furthermore, soil microbial communities co-exist with other soil fauna communities in the soil food web, competing for resources and being preyed on by fauna at higher trophic levels. More importantly, such interactions likely further regulate the availability of soil nutrients for plants. However, our understanding of the soil food web interactions is still limited, particularly as to how the interactions influence the development and growth of plants.

The interaction between the soil bacterial and fungal communities and the soil nematode community is important and ubiquitous in the soil food web, which can have implications at an agroecosystem level. This is due to i) the high abundance of the soil bacterial and fungal communities, with their biomass being

10^2 to 10^4 times greater than other soil microfauna[3]; ii), nematodes communities being the most abundant soil animals[4]; and iii), multiple preferences in feeding strategies within the nematode community, including free living bacterial feeders, fungal feeders and omnivores as well as plant pathogens[4,5]. Due to these multiple feeding preferences, soil nematode communities build an additional link between plant root and soil microbial communities. Such interconnection between soil microbes-nematodes and roots may potentially alter the availability and uptake of plant nutrients, and eventually plant growth and development. For example, the introduction of different nematode species into a soil induced strong interaction with soil microbial communities, during which soil bacterial biomass and N mineralisation rate were increased, and soil bacterial community composition was also altered[6]. Similarly, the predation of nematodes on soil microbial community promoted soil carbon metabolic activities[7]. However, we still lack fundamental understanding as to how plant growth responds to such interaction, despite the significant changes in soil nutrients mineralisation rate in previous studies[6-8].

In this study, we tested the response of wheat and canola to the interaction between soil microbial community and nematode community via real-time monitoring of plant responses through non-destructive plant growth measurements in The Plant Accelerator (TPA) at The University of Adelaide. The findings bring novel insights on the regulation of the soil food web interactions on crop growth and performance, and shed light on how to harness the biological functions of both soil microbes and nematodes towards future sustainable and productive farming systems.

Materials and Method

Soils and crop varieties

Agricultural soil from broadacre paddocks in Paskeville on the Yorke Peninsula (hereafter abbreviated as 'YP') and Cummins on the Eyre Peninsula ('EP') in South Australia, and in Charlton near Birchip ('Birchip') in Victoria were collected and used for the experiments. Wheat variety 'Scepter' (*Triticum aestivum*) and field pea 'Wharton' (*Pisum sativum*) were used due to their importance in broadacre farming in South Australia and nationally. Both crops were grown for six weeks in a Smart house at TPA with the shoot biomass and growth rates of individual plants non-destructively monitored by red-green-blue (RGB) imaging during weeks 3 to 6 after sowing. After harvesting, plant root systems were extracted from the soil and dry weight was recorded. A soil sample per pot was also collected after harvest and sent to SARDI Molecular Diagnostic Centre for soil DNA extraction and estimation of free living and plant pathogenic nematode populations using quantitative PCR (qPCR). DNA was also used for sequencing of both soil bacterial and fungal communities by the Australian Genome Research Facility (AGRF) to determine dynamic changes in the number of both soil microbial and nematodes communities.

Experimental design and preparation

All soils were passed through a 2mm sieve and large gravel and stubble were removed. Following the method by Wagg et al.[9] (personal communication), we developed a protocol to separate the soil microbial community and nematode community using wet sieving on a 25μm sieve. The separated microbial and nematode community were verified using qPCR, and were added back into the soil as inoculant. The soil sterilisation was done by two rounds of autoclaving at 121^{o}C at 1-2 week interval. Experimental treatments included a sterilised control treatment without any addition, or sterilised soil re-inoculated with either microbial or nematode community, or the combination of both communities. A standard control treatment using unsterilised soil were also included. In total, the experiment comprised 5 soil treatments * 3 soil types * 2 crop types (wheat and canola) * 8 replicates (240 pots). During the experiment, all pots were arranged using a split-unit design by allocating 16 blocks each comprising 3 Lanes × 5 Positions on the conveyer belt

at TPA. Each experimental block was divided into 3 main units by soil types, each consisting of five consecutive carts in the same lane. The soils were randomised to the main units and five treatments within a main unit were also randomised. The design was constructed and randomised using R package 'dae' [10], a package for the R statistical computing environment [11].

Results

The separation of soil microbes and nematodes

The two rounds of wet sieving efficiently separated the soil microbial community and the nematode community for all soil types. Nearly no free-living nematode DNA was detected in the <25μm fraction from all soils compared to the >25μm fraction, with 1, 0 and 4 kDNA copies per g of soil vs 230, 247 and 588 kDNA copies detected in Birchip, EP and YP soil, respectively (Table 1). Plant pathogenic nematodes (*Pratylenchus neglectus*) were also only detected in the >25μm fraction in YP soil.

The response of wheat and canola to the addition of soil microbial and nematode communities

All pots were placed on the imaging conveyer belt 15 days after planting (DAP). For wheat, large differences were found in smoothed projected shoot area (sPSA) between unsterilised and sterilised treatments in Birchip and YP soil from 16 DAP onwards, with the magnitude of difference becoming larger toward the end of the experiment (Figure 1a). For example, sPSA was 26% and 30% larger in sterilised than unsterilised treatment at 16 DAP in Birchip and YP soil, and increased to 83% and 52% at 43 DAP, respectively. In EP soil, plants from the unsterilised treatment died due to damping-off, while plants in the treatments in sterilised soil were not affected, but no difference was found between treatments.

For canola, there were marked differences from 33 DAP onwards for Birchip and YP soils between unsterilised treatment and the four sterilised treatments (Figure 1b). In EP soil, plants in the unsterilised control soil also died due to damping-off. The differences between the four sterilised treatments were smaller, but significant for some soils. For example, in Birchip and EP soils, the addition of microbes and/or nematodes increased sPSA from DAP 26, compared to the sterilised treatments. In YP soil, only the addition of both microbes and nematodes increased sPSA from DAP40, compared to the addition of microbes alone. Most of the other differences between the four sterilised treatments are nonsignificant. In EP soil, the addition of microbes was significantly better than adding both microbes and nematodes at 40 DAP.

Conclusion

Our study provides strong evidence on the effect of soil food web interactions on plant development over time, highlighting the role of complex interconnections between soil microbial community and nematode community in regulating plant growth. The significant difference in plant shoot growth and development between unsterilised and sterilised soil is currently being examined, while the difference between crop types will be further investigated in another project.

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Table 1. The qPCR estimation of free-living nematode and plant pathogenic nematode DNA concentrations in the <25um (microbial community) and >25um (nematode community) fractions obtained by wet sieving of soils collected in Birchip, Eyre Peninsula (EP) and Yorke Peninsula (YP). DNA concentrations are presented as kDNA copies per gram soil for free living nematodes and as equivalent nematodes per gram of soil for the two *Pratylenchus* species (*P. neglectus* and *P. thornei*) or eggs per gram of soil for the Cereal cyst nematode (CCN).

Soil	Fractions	Free-living nematodes (kDNA copies/g soil)	Pathogenic nematode species		
			P. neglectus (nematodes/g soil)	*P. thornei* (nematodes/g soil)	CCN (egg/g sample)
Birchip	>25µm	230	0	0	0
EP	>25µm	247	0	0	0
YP	>25µm	588	31	0	0
Birchip	<25µm	1	0	0	0
EP	<25µm	0	0	0	0
YP	<25µm	4	0	0	0

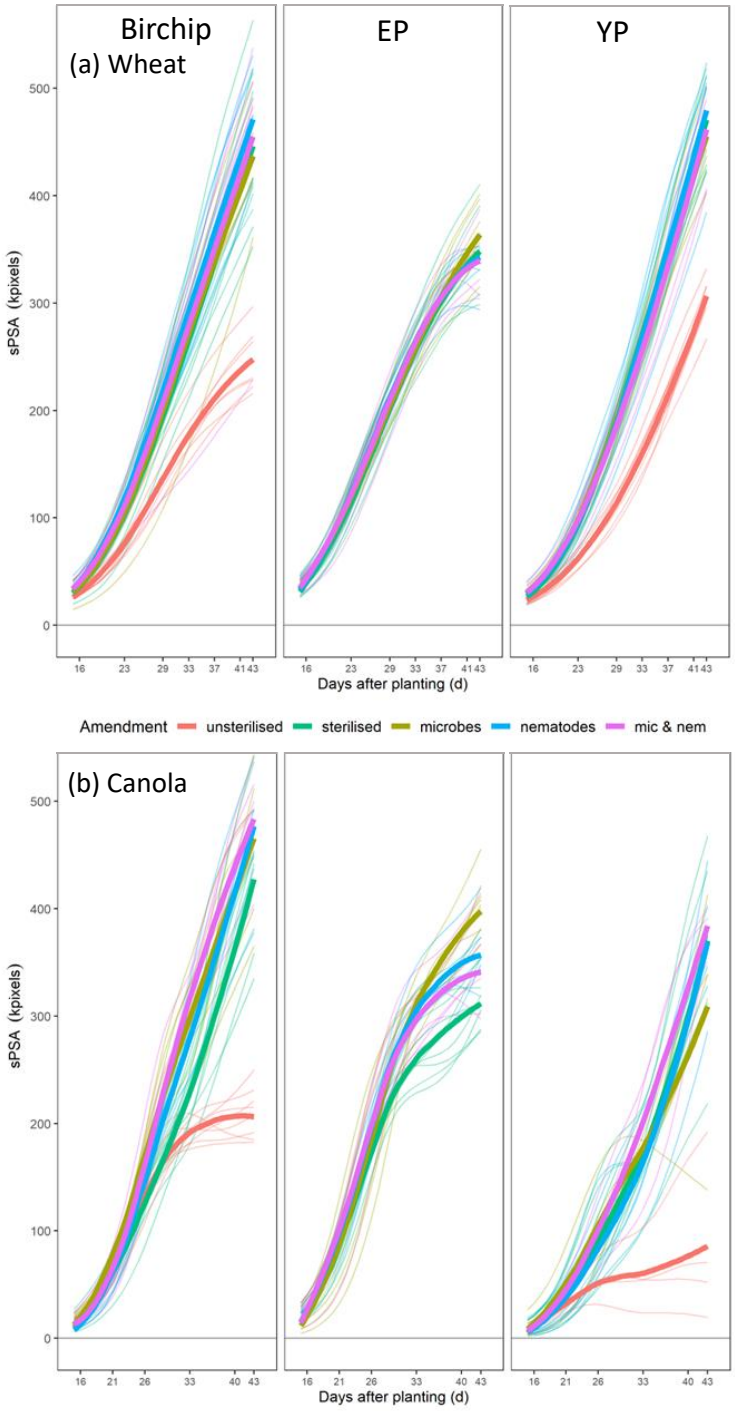

Figure 1. Smoothed projected shoot area (sPSA) of wheat (a) and canola (b) over days after planting (DAPs) for three soils (Birchip, EP and YP) under five treatments, including unsterilised, sterilised, sterilised plus microbial community, sterilised plus nematode community and sterilised plus microbial and nematode communities. Each curve corresponds to one plant, and the thickened curve represents the smooth regression loess curve fit for each of the treatments.