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Allelopathic Root Leachate Effects of *Lolium multiflorum x L. perenne* on Crops and the Concomitant Changes in Metabolic Potential of the Soil Microbial Community as Indicated by the Biolog Ecoplate™

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MIF, CFR and AB designed the study. Author MR performed the statistical analysis. Author MIF wrote the protocol and wrote the first draft of the manuscript. Authors MIF, AM and AB managed the analyses of the study. Authors MIF and AM managed the literature searches. The manuscript was edited by authors CFR and AB. All authors read and approved the final manuscript.

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ABSTRACT

Plant roots serve a multitude of functions including anchorage, provision of nutrients and water, as well as production of exudates with growth regulatory properties. Some root exudate components may act as allelochemicals and mediate interactions between plants and other organisms in the rhizosphere. The significance of micro-organisms in influencing allelopathic activity is largely not

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investigated in bioassays for allelopathy. The aim of this study was to test the appropriateness of Biolog EcoPlates™ as a quick and relatively cheap method of establishing the presence of microbe interactions mediated by allelopathic pot leachates from either rotational crops or *L. multiflorum x L. perenne*. In this green house study, Biolog EcoPlates™ were used to indicate the effect of pot leachates from six different donor plants on the soil microbial populations associated with the same species serving as acceptor plants, grown in pots with soil from the same origin in both sets of pots. Pot leachates from donor plants were added to the acceptor plants on a weekly basis until plants reached maturity. Soil samples from acceptor pots were used to inoculate Biolog EcoPlates™ and the carbon utilisation patterns were compared to the pattern obtained for the soil microbial populations before treatment commenced. Findings indicate that root exudates can influence the microbial community structure in the rhizosphere and location is an important factor governing plant-plant and plant-microbe interactions. The Biolog EcoPlate™ could be used as an indicator of the allelopathic activity of crop or weed species.

Keywords: Allelochemicals; micro-organisms; rhizosphere; rotational crops.

1. INTRODUCTION

Plant roots serve a multitude of functions including anchorage, provision of nutrients and water, as well as production of exudates with growth regulatory properties. The root-soil interface, or rhizosphere, is the site of greatest activity within the soil matrix since all roots have the ability to secrete chemical compounds in response to biotic and abiotic stresses [1]. These root exudates may comprise of a wide variety of metabolites including carbohydrates, proteins, vitamins, amino acids, and other organic compounds [2].

Root exudation serves as an important carbon and energy source for micro-organisms contained in the rhizosphere [3]. Also some of the low molecular weight root exudate components may also act as allelochemicals and mediate interactions between plants and other organisms in the rhizosphere [1]. These allelochemicals may impact on weed and pest populations as well as on diseases [1]. Today we know that allelopathic processes involve secretion of bioactive compounds from both plants and micro-organisms resulting in the inhibition or stimulation of physiological processes in neighbouring individuals belonging to either the same or different species [4,5,6]. According to [4], the term allelopathy has therefore been broadened, to include not only plant-to-plant, but also plant-to-micro-organism interactions. However, soil micro-organisms may limit allelopathic expression once chemicals are released into soil [7]. Thus, after their entry into the soil environment, persistence, availability and biological activities of allelochemicals are influenced by micro-organisms [8].

It had been proven by [6] that allelopathic rice release allelochemicals from roots into soil at significant rates to interact with soil micro-organisms. It was stated that potent allelochemicals from the rice material and root exudates may modify soil micro-organisms to the crop's advantage [9]. Further findings made by [9] imply that soil microbial populations are affected by the compounds released by allelopathic rice varieties. However, the mere presence of chemicals in the donor plant and its phytotoxic activities in an artificial medium does not demonstrate its allelopathic activity in natural situations [7], because one of the shortcomings of many bioassays for allelopathy is the absence of soil or the employment of an artificial growth medium such as agar [10]. Also, the significance of micro-organisms in influencing allelopathic activity is largely not investigated in bioassays for allelopathy [7]. The soil microflora might stimulate root exudates [11]. However, the laboratory "rhizospheres" are not an adequate surrogate for real soil environment [12]. However, since there are interactions among plants, microbes, as well as plants and microbes, in the soil environment, it was decide to use pot leachates from donor plants in unsterilised soils in a greenhouse experiment, to simulate field conditions as closely as possible.

Soil microbes have functional interactions with their environment and therefore it is important to analyse the microbial communities in the soil as a single ecological unit [13]. Molecular methods are often used to measure changes in microbial communities [14,15]. However, it was found [16] that the culturable microbe fraction of the soil community is often that which acts as indicator to soil disturbances. It seems that this fraction

contribute the most to the functionality of the ecosystem [16]. Soil microbial community analyses were conducted by a number of workers using the Biolog™ system with variable success [17,18,19]. The Biolog Ecoplate™ enables the description of microbial communities in ecologically relevant terms. This methodology introduced [17] to visualise the physiological traits of microbial communities, during both the characterising of the community functional attributes and assessing community dynamics. In applied ecological research, the Biolog EcoPlate™ is used as both an assay of the stability of a normal population and to detect and assess changes based upon the variables that are introduced (www.biolog.com). The Biolog EcoPlate™ presents micro-organisms in the soil solution with 31 different carbon sources. The consumption of these carbon sources would be specific to a microbial community, presenting the observer with a physiological profile of the microbial community under observation. Any changes in the composition of this microbial community will thus be reflected in changes in the carbon source utilisation pattern.

The Biolog EcoPlate™ system is not often used for soil microbial community analyses after treatment with weed root leachates. Nevertheless, [19] were able to use Biolog EcoPlates™ to distinguish between sites contaminated by the plant invader *Solidago canadensis* and natural sites, while [20] indicated different carbon ustilisation patterns associated with different plant species. Consequently, it was important to test its appropriateness as a quick and relatively cheap method of establishing the presence of microbe interactions mediated by allelopathic root leachates from either rotational crops, including wheat, or the weed *L. multiflorum x L. perenne* [21]. Very little is known about this weed hybrid, which easily develops herbicide resistance and its invasive mechanism in which allelopathic and microbial characteristics are thought to play a crucial role. Therefore, a description of the effect of *L. multiflorum x L. perenne* root leachates on soil microbes would be very important in planning control strategies against this weed. To address this lack of knowledge, the objective of this study was to assess the pot leachate effects of crops and a weed in two soil types representative of important crop rotational systems of the Western Cape Province. We hypothesised that pot leachates of donor plants in our study will have a definite influence on the functional soil microbial community of acceptor plants. To this end, using the Biolog EcoPlate™ system, concomitant changes in the metabolic potential of the soil microbial community were studied for all the pot leachate interactions that were investigated.

2. MATERIALS AND METHODS

2.1 Pot Experiment

The plant series used in a greenhouse study comprised the rotational crops barley (*Hordeum vulgare* L. v. Clipper), wheat (*Triticum aestivum* v. SST 027), lupines (*Lupinus albus* L. v. Tanjil and v. Quilinock), rye grass (*Lolium multiflorum* Lam. v. Energa) and the weed type rye grass *(L. multiflorum x L. perenne).*

The research approach was based on research methods followed by [22,23,24] for assessing whether crop root exudates release phytotoxins that affect the growth and yield of rotational crops and weeds. The present study was however different in terms of both experimental method and plant series investigated.

Topsoil from two diverse localities, namely Langgewens (18°70'E, 33°27'S) and Tygerhoek (19°54'E, 34°08'S) research farms of the Western Cape, was collected for a greenhouse experiment. Soils from Langgewens are residual (pH 6.3) and of Glenrosa (Entisol) type [25]. Tygerhoek soils are weakly developed residual soils (pH 5.2) and of Mispah (Entisol) type. In the greenhouse, which was set at a constant temperature of 18°C, natural lighting was used, simulating normal day length for the crop growth period from May to October (Southern hemisphere).

Experimental design made provision for the establishment of "donor" plants in pots from which leachates were collected on a regular basis to treat "acceptor" plants grown in separate pots. Each pot (diameter 17 cm; depth 20 cm) was filled with 6 kg of mixed soil collected from either Langgewens or Tygerhoek. For both the "donor" and "acceptor" plant series, six crop seeds of each plant type were planted in potted soil during May. Seedlings were thinned to three plants of similar size one week after emergence. At two weeks after planting, 0.1 g N in the form of limestone ammonium nitrate (LAN) was added to all pots containing Graminaceae species. Once a week, 100 ml Multifeed (Plaaskem (Pty) Ltd, Witfield, South Africa) was applied as a balanced plant nutrition solution at a concentration of 1 g ℓ^{-1}, to each pot for the first four weeks and

thereafter fortnightly. Each donor pot was over-irrigated bi-weekly with 200 ml tap water from the first week after planting to ensure drainage from pots. In the case of the “donor” series all water leached from the same plant type was collected in a single container and used as pot leachate treatment for a particular receptor plant. No planting was done in control pots, but the leachate was collected in the same way as described above for use as control treatment. Treatments in the greenhouse were replicated three times in a randomised block design and the experiment was repeated once.

Of the leachate collected from the “donor" plant series, which served as sources of allelochemicals, 150 ml was transferred bi-weekly to the “acceptor” plant series. In this way the leachate from a particular species was applied to plants of the same type as well as to each of the other plant types. The first transfer of leachate took place at the time of planting, and thereafter bi-weekly up to sixteen weeks after emergence. The retention time of the volume of leachate in the containers in which it was collected from the “donor” plants and from which it was dispersed to the “acceptor” plants, was one hour.

2.2 Microbial Community Analysis

To determine changes in microbial populations over the trial period, whole community metabolic analyses were performed on both soils used in the pot experiment at the onset and end of the experimental period [17].

Soil samples of 10 g each were taken before filling of the pots to serve as reference point. After harvesting of plants, two soil samples of 10 g were again taken from each treatment. All soil samples taken in this way were suspended in 90 ml sterile distilled water. After shaking for 10 minutes the sample was prolapsed and inoculated directly into Biolog EcoPlate™ (Biolog, Haywood, CA, USA) as a soil suspension and incubated at 22℃ in the dark. After 48 hours the microbial community-level physiological profile was assessed for colour development. Utilisation of the carbon source in each well, indicated by a reduction of the tetrazolium dye, was then recorded as either negative (carbon source not used) or positive (carbon source used). The utilisation of a carbon source (positive reaction), was indicated by a colour change when compared to the control without any carbon source.

2.3 Plant and Microbial Data Collection and Statistical Analysis

Plant height was determined for all acceptor plants on a weekly basis, starting from the first week after planting until plants were harvested at maturity. Plants were regarded as mature when the reproductive growth phase was completed at the onset of senescence as indicated by visible loss of chlorophyll, i.e. yellowing of leaves. Growth rate was measured and expressed as cm gained per day from the regression parameters of the fitted regression models. Because of differences in plant growth patterns between the two localities, data for each soil type were analysed separately. All data were averaged over the two sets of data for each locality and were analysed statistically (ANOVA) with the statistical program SAS. Least significant difference (LSD) values were used to differentiate between the effects of the donor plant series on the acceptor plant series at the 5% level of probability.

The carbon-source-use Biolog EcoPlate™ data, collected on the two sampling occasions were analysed using principal component analysis (PCA) to determine the effects of pot leachate treatments on soil micro-organisms. PCA was done with Pearson correlation matrix as input. Principal Components Analysis (PCA) provides a concise overview of a dataset and is very powerful at recognising patterns in data. The score plot shows how treatments relate to each other based on microbe activity and logistic growth curve parameters. Treatments with similar profiles lie close to each other on the map while treatments with different profiles lie far apart. Comparing the score and loading plot can identify the relationships between treatments and variables.

3. RESULTS

3.1 Growth Rates of *H. vulgare L.* v. *Clipper, T. aestivum, L. albus v. Tanjil* and *L. albus* v. Quilinock

It was evident from the results on growth rate that effects from pot leachates were more pronounced on Langgewens than on Tygerhoek soils. For *H. vulgare, T. aestivum, L. albus v*. Tanjil and *L. albus v.* Quilinock grown on Tygerhoek soil, no significant differences in growth rate were recorded following treatment with pot leachates (data not presented).

The growth rate of *H. vulgare* grown on Langgewens soil and exposed to *H. vulgare* or *L. albus* v. Tanjil pot leachates was significantly greater than the control (zero pot leachates) (Table 1). *H. vulgare* grown on the same soil and treated with *T. aestivum, L. albus* v. Quilinock, *L. multiflorum* v. Energa or *L. multiflorum x L. perenne* pot leachates had its growth rate reduced compared to the control (Table 1). *L. albus* v. Tanjil or v. Quilinock pot leachates caused a significant increase from the control in *T. aestivum* growth rate, when grown on Langgewens soil.

L. albus v. Tanjil, grown on Langgewens soil and exposed to *L. albus* v. Quilinock pot leachate, had a significantly higher growth rate than that attained at the control (Table 1). The growth rate of *L. albus* L. v. Quilinock grown on Langgewens soil and exposed to *H. vulgare, T. aestivum* or *L. multiflorum x L. perenne* pot leachates was significantly greater than the control.

3.2 Score and Loading Plots of *H. vulgare* L. v. Clipper, *T. aestivum, L. albus* v. Tanjil *and L. albus* v. Quilinock

Pot leachates had a significant impact on the metabolic potential of the soil microbial community. From the loading plots (Figs. 1 – 3) it is clear that carbon sources, of which the utilisation increased concomitantly with enhanced plant growth rate, differed between plant species and soil types. Furthermore, score plots for both Graminaceae and *Lupinus* spp. show similar physiological profiles for locality and plant family, indicating similar micro-organism populations with similar function in the rhizosphere for grain and leguminous crops, respectively.

In the score plot for *H. vulgare* grown on Langgewens soil, a physiological profile was observed which clustered together in the top left quadrant, showing a correlation with growth rate which had an association with D-Mannitol (line 14) as carbon source. The loading plot indicates that utilised carbon sources which clustered together in the top left quadrant followed treatments with pot leachates from *L. albus v*. Tanjil, *L. albus v.* Quilinock or *L. multiflorum x L. perenne* (Fig. 1a).

For *H. vulgare* grown on Tygerhoek soil, carbon source utilisation was observed in the top right quadrant of the score plot in Fig. 1b, correlating with growth rate and associated with L-Arginine, Pyruvic Acid Methyl Ester, D-Xylose, α-Cyclodextrin, y-Hydroxibutyric Acid, Itatonic Acid, α-D-Lactose and D,L-α-Glycerol Phosphate as carbon sources (lines 4, 5, 6, 17, 19, 23, 29 and 30). The top right quadrant of the loading plot indicates that microbes utilizing the aforementioned carbon sources were affected by *T. aestivum* pot leachates (Fig. 1b).

In the score plot of Fig. 2a, the physiological profile for *T. aestivum* grown on Langgewens soil, clustered in the bottom right quadrant which shows a correlation with growth rate and an association with D-Galacturonic Acid, L-Serine, y-Hydroxibutyric Acid and Phenylehthylamine as carbon sources (lines 7, 16, 19 and 28). The bottom right quadrant of the loading plot reveals that this followed treatment with *H. vulgare* pot leachates (Fig. 2a).

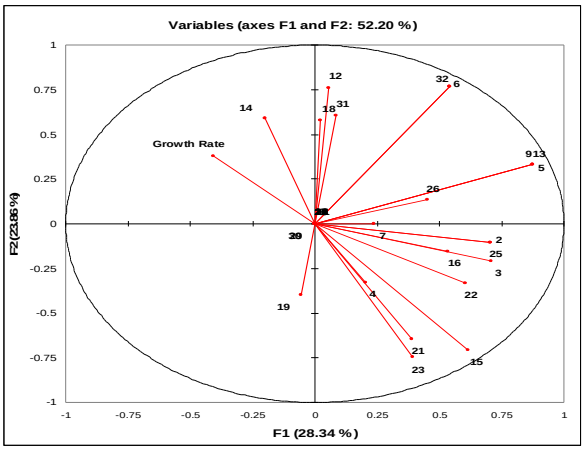
The score plot in Fig. 2b indicates that a cluster of utilised carbon sources in the bottom left quadrant correlates with growth rate and is associated with 2-Hydroxy-Benzoic Acid, Itatonic Acid, α-D-Lactose and D,L-α-Glycerol Phosphate as carbon sources (lines 11, 23, 29 and 30). This followed treatment of *T. aestivum* grown on Tygerhoek soil, with *L. albus v*. Tanjil pot leachates, as revealed by the loading plot.

The score plot for Langgewens soil for *L. albus* v. Tanjil (data not presented) indicates that the physiological profile which clustered together in the top right quadrant has a correlation with growth rate and an association with L-Arginine, α-Cyclodextrin, D-Glucosaminic Acid, Glucose-1-Phosphate and α-D-Lactose as carbon sources. This corresponds with the physiological profile clustering together in the top right quadrant of the loading plot, following treatment of *L. albus* v. Tanjil, grown on Langgewens soil and treated with *L. albus* v. Tanjil, *L. albus* v. Quilinock, *L. multiflorum* v. Energa or *L. multiflorum x L. perenne* pot leachates.

The score plot for Tygerhoek soil for *L. albus* v. Tanjil reveals a physiological profile, which clustered together in the top right quadrant; correlating with growth rate and associated with L-Arginine, α-Cyclodextrin, D-Glucosaminic Acid and Glucose-1-Phosphate as carbon sources (lines 4, 17, 22, 26 and 29). The top right quadrant of the loading plot indicates that microbes utilizing those five carbon sources were not affected by any pot leachates.

Table 1. Effects of pot leachates from the donor plant series on growth rate of *Hordeum vulgare* L. v. Clipper, *Triticum aestivum* v. SST 027, *Lupinus albus v.* Tanjil and *Lupinus albus v.* Quilinock on Langgewens soils

Plant type	*H. vulgare*	*T. aestivum*	*L. albus v.* Tanjil	*L. albus* v. Quilinock
	Growth rate X 10^{-2} cm day^{-1}	Growth rate X 10^{-2} cm day^{-1}	Growth rate X 10^{-2} cm day^{-1}	Growth rate X 10^{-2} cm day^{-1}
H. vulgare L. v. Clipper	5.575a	5.435ab	5.366b	5.073ab
T. aestivum v. SST 027	4.405c	5.466ab	4.789b	5.656a
L. albus L. v. Tanjil	5.931a	5.813a	5.831ab	4.665bc
L. albus v. Quilinock	4.153c	5.734a	6.634a	4.937bc
L. multiflorum v. Energa	4.209c	4.987bc	4.930b	4.372c
L. multiflorum x perenne	4.365c	4.765c	5.671ab	5.243ab
Control	4.996b	5.109bc	5.482b	4.467c
LSD (P=0.05)	0.410	0.500	1.100	0.600



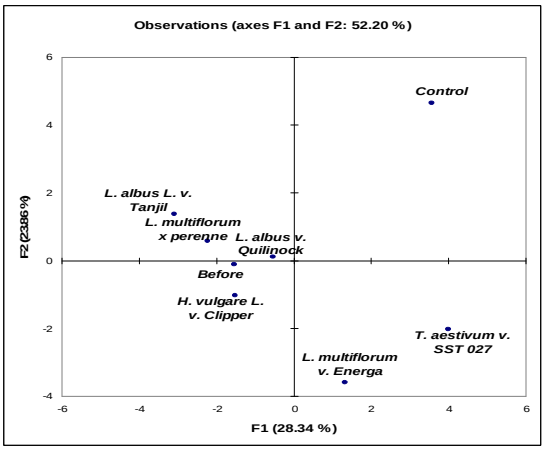
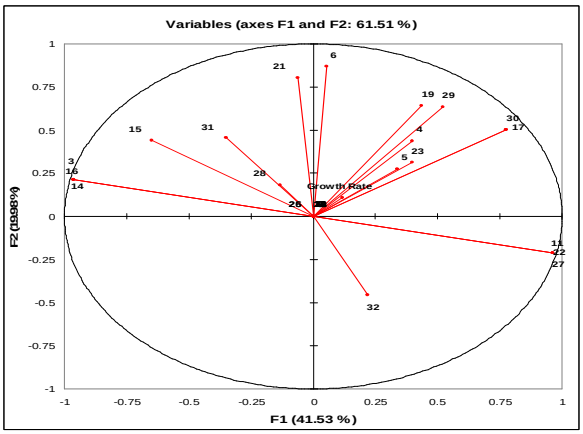


Fig. 1a. Score plot (left) and loading plot (right) of *Hordeum vulgare* v. Clipper grown on Langgewens soil, and its association with soil micro-organisms



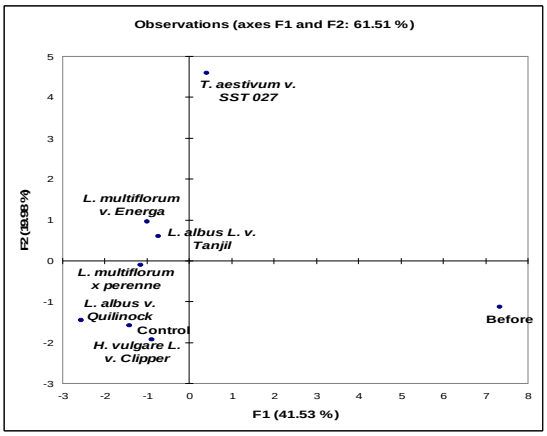


Fig. 1b. Score plot (left) and loading plot (right) of *Hordeum vulgare* v. Clipper grown on Tygerhoek soil, and its association with soil micro-organisms

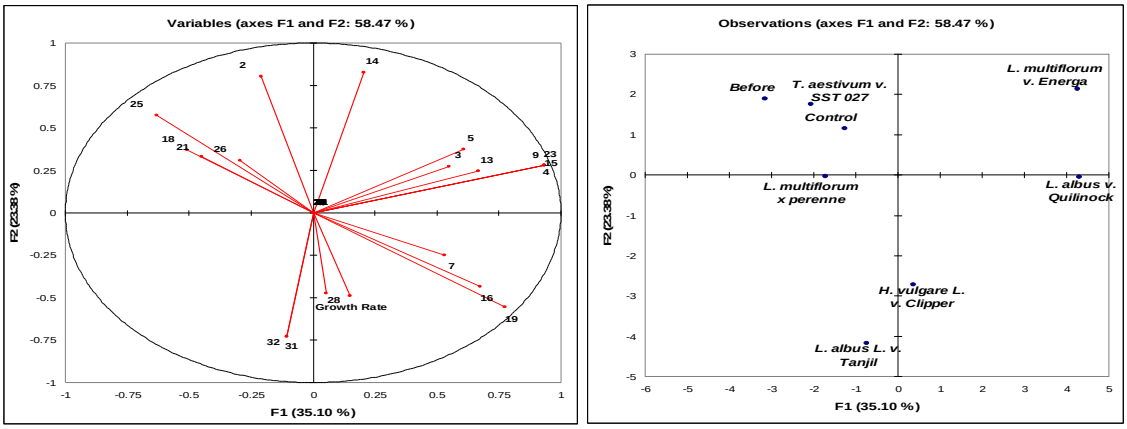


Fig. 2a. Score plot (left) and loading plot (right) of *Triticum aestivum* v. SST 027 grown on Langgewens soil, and its association with soil micro-organisms

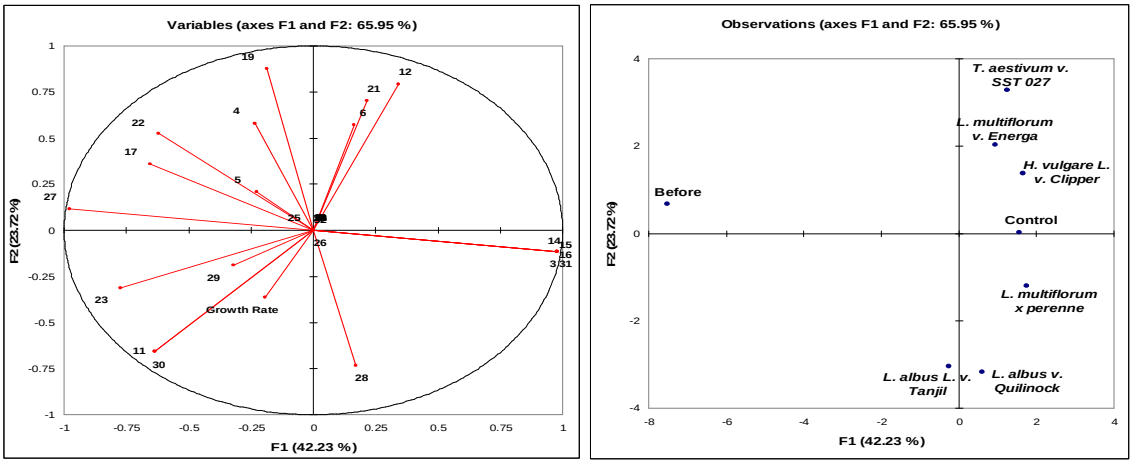


Fig. 2b. Score plot (left) and loading plot (right) of *Triticum aestivum* v. SST 027 grown on Tygerhoek soil, and its association with soil micro-organisms

The physiological profile in the score plot of *L. albus* L. v. Quilinock, which clustered together in the bottom right quadrant, indicates a correlation with growth rate which had an association with D-Xylose, Tween 80, Glycyl-L-Glutamic Acid, Phenylehthylamine, D-Mallic Acid and Putrecine as carbon sources. The loading plot indicates that treatment of *L. albus* L. v. Quilinock grown on Langgewens soil, with pot leachates from *L. albus* v. Tanjil, *L. albus* L. v. Quilinock or *H. vulgare,* resulted in this cluster of carbon source utilisation in the bottom right quadrant.

In the score plot for *L. albus* v. Quilinock grown on Tygerhoek soil and treated with *L. albus* v. Quilinock or *L. multiflorum v.* Energa pot leachates, a profile of carbon sources was observed as it clustered together in the bottom left quadrant, indicating a correlation with growth rate which had an association with I-Erythritol, L-Phenylalanine, Glycyl-L-Glutamic Acid and Phenylehthylamine as carbon sources (lines 10, 12, 24 and 28). The bottom left quadrant of the loading plot reveals that this followed treatment with *H. vulgare* pot leachates.

3.3 Growth Rate of *L. multiflorum* v. Energa *and L. multiflorum x L. perenne*

H. vulgare pot leachate significantly inhibited the growth rate of *L. multiflorum* v. Energa grown on Langgewens soil (Table 2). The growth rate of *L. multiflorum* v. Energa grown on Tygerhoek soil and treated with *L. multiflorum* v. Energa pot leachate, was significantly higher than the control (Table 2).

The growth rate of *L. multiflorum x L. perenne* grown on Langgewens soil and treated with *H. vulgare* pot leachates, was highly significantly (P=0.01) higher, while *T. aestivum* or *L. multiflorum x L. perenne* pot leachates, was significantly (P=0.05) higher than the control (Table 2). No significant differences between the control and other treatments were observed in the growth rate of *L. multiflorum x L. perenne* grown on Tygerhoek soil (Table 2).

3.4 Score and Loading Plots of *L. multiflorum* v. Energa *and L. multiflorum x L. perenne*

The score plot for Tygerhoek soil in Fig. 3 indicates that utilised carbon sources which cluster together in the top left quadrant had a correlation with growth rate and an association with L-Arginine, Tween 40, α-Cyclodextrin, N-Acetyl-D-Glucosamine, y-Hydroxibutyric Acid, Itatonic Acid and α-D-Lactose as of carbon sources. The top left quadrant of the loading plot indicates that microbes utilizing those carbon sources were not affected by any pot leachates (Fig. 3).

Score plots for *L. multiflorum x L. perenne* grown on both Langgewens and Tygerhoek soils indicates that treatment with all pot leachates had no effect. Loading plots for both *Lolium* spp. however, show similar physiological profiles at each locality for both species, indicating similar micro-organism populations in the rhizosphere for this genus (data not presented).

Table 2. Effects of pot leachates from the donor plant series on growth rate of *Lolium multiflorum* v. Energa and *Lolium multiflorum x Lolium perenne* on Langgewens or Tygerhoek soils

Plant type	*L. multiflorum* v. Energa Growth rate X 10^{-2} cm day^{-1}		*L. multiflorum x perenne* Growth rate X 10^{-2} cm day^{-1}	
	Langgewens soil	Tygerhoek soil	Langgewens soil	Tygerhoek soil
H. vulgare L. v. Clipper	6.385c	5.009b	3.331a	2.399a
T. aestivum v. SST 027	6.940a	4.894bc	3.019b	2.240b
L. albus L. v. Tanjil	7.115a	4.570c	2.823c	2.289ab
L. albus v. Quilinock	7.206a	4.637bc	2.883c	2.375a
L. multiflorum v. Energa	6.484bc	5.390a	2.768c	2.294ab
L. multiflorum x perenne	6.445bc	5.002b	3.132b	2.290ab
Control	6.848ab	4.902bc	2.829c	2.341ab

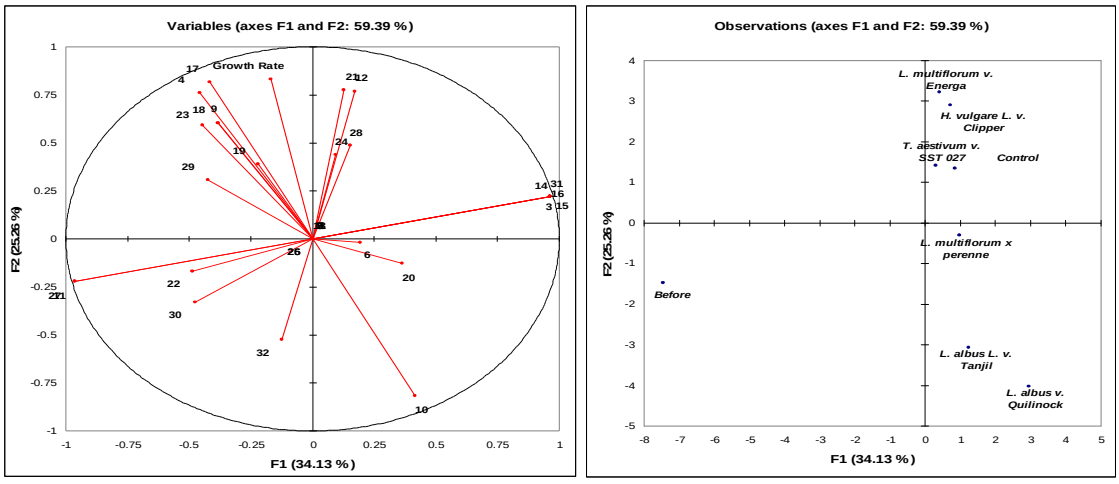


Fig. 3. Score plot (left) and loading plot (right) of *Lolium multiflorum* v. Energa grown on Tygerhoek soil, and its association with soil micro-organisms

4. DISCUSSION

Results imply that soil microbial populations are affected by the compounds released by the different plant types and the composition of their pot exudates. In terms of growth rate this may be due to the fact that pot exudates released from plant seedlings grown on Langgewens soil stimulate soil microbial populations in the rhizosphere more so than those grown on Tygerhoek soil. It appeared therefore that the soil microbial populations at Langgewens were different from those at Tygerhoek. These findings correspond with those reviewed by [1] that roots release leachates containing allelochemicals at significant rates to soil micro-organisms and that soil micro-organisms are an important determinant of allelopathic activity [7].

In this study we showed that by releasing leachates from their roots, different plant types may have a great impact on microbial population and community structure in the two soil types studied. Furthermore, pot leachates had a significant impact on the metabolic potential of the soil microbial community. Also, carbon sources, of which the utilisation increased concomitantly with enhanced plant growth rate, differed between plant species and soil types. It was also reported [26] that the components of rice root exudates could affect soil-borne microbes.

4.1 *H. vulgare* L. v. Clipper

The growth rate of *H. vulgare* was increased by pot leachates from *H. vulgare*, and slowed by those from *L. albus* v. Quilinock. Principal component analysis (PCA) indicated that soil micro-organisms responded differently to those treatments, which may or may not influence allelochemical bioactivity and/or plant growth. Both [27] and [28] reported inhibitory effects by *L. albus* on crop plants. In addition, [29] reported that aqueous extracts of many weed species inhibited germination, coleoptile length, root length, and shoot and root dry weight of barley seedlings grown in Petri-dishes. Barley was found to be one of the most susceptible crops to phytotoxic residue extracts from the grass weed *Vulpia myuros* [30]. Water extracts from fresh roots of *Cardaria draba* inhibited wheat and barley root length by 11 and 33.3% and root dry weight by 17 and 53%, respectively. This was confirmed by [31] who reported on the reduction in barley growth rate when exposed to extracts from different barley cultivars, hence indicating autotoxicity. However, it was emphasised by [32] that the concentration span between stimulation and inhibition for allelochemicals can be small and hormetic effects may occur in a natural setting if doses released are low.

It was suggested [33] that different rice cultivars have different selectivity against weed species, indicating that several chemicals are involved in allelopathic action. Broadleaved and grass plants differ in sensitivity towards particular allelochemicals. It should be borne in mind that different secretion rates of the same allelochemicals could have resulted in different growth responses from the species considered here. This dose/response phenomenon is termed hormesis and represents an evolutionarily conserved process of adaptive, potentially beneficial responses to low doses of a stressor agent [34]. Dose/response studies showed that the occurrence and the magnitude of hormesis depended on concentration of the allelochemical, climatic conditions and the parameter measured [32]. Furthermore, as mentioned earlier, the span between stimulation and inhibition for allelochemicals can be small and hormetic effects may occur in a natural setting if doses released are low [32]. Under field conditions this equates to higher and lower doses as plant density varies.

4.2 *T. aestivum* v. SST 027

On Langgewens soil, the growth rate of wheat was stimulated by *L. albus* v. Tanjil or *L. albus* v. Quilinock. This significantly faster growth rate of wheat can most probably be attributed to the N fixing ability of lupines, as N compounds are known to stimulate growth of many plant species [35]. Any combined chemical root exudates, including allelopathic effects of a stimulatory nature, could have been masked by the growth promoting effect of nitrogen that conceivably was added to the system by the legume.

An association with microbes utilising particular carbon sources was indicated by PCA, when treated with pot leachates from *L. multiflorum* v. Energa or *T. aestivum,* respectively. Root exudation serves as an important carbon and energy source for micro-organisms contained in the rhizosphere [1]. Therefore, it is conceivable that soil microbial populations used particular carbon sources which influenced the growth rate of wheat grown on either Langgewens or

Tygerhoek soils. It was confirmed [9] that variation of the soil microbial populations and community structures could be distinguished by the allelopathic and non-allelopathic crop varieties tested. Although the present study did not consider only the effects of allelochemicals contained in root leachates, but the combined effects of all solutes contained in them, it was indicated that the effect on soil microbial population and community structure may be pronounced. This corresponds with the findings of [9] that the composition of soil microbes is defined at least in part by the nature and amount of chemicals contained in pot exudates. Therefore, we contend that the growth rate of test plants in this study could be ascribed to the combination of compounds contributed by pot exudates and soil microbial populations. Furthermore, differences in plant growth rate and responses in physiological profiles of micro-organisms observed on the two soils used in the study, suggest that location is an important factor governing plant-plant interactions.

4.3 *L. albus* L. v. Tanjil

The faster growth rate of *L. albus* v. Tanjil, grown on Langgewens soil when exposed to *L. albus* v. Quilinock pot leachate was probably associated with soil micro-organisms and not plant-derived allelopathic compounds. Results [9] showed that crop varieties could modify soil microorganisms to their advantage through the release of allelochemicals. Roots lose allelochemicals to the soil at rates of significance to interact with soil microorganisms [1]. In turn, soil microorganisms consume and decompose allelochemicals and are an important determinant of allelopathic activity [36]. Microbial degradation of a particular allelochemical depends upon the specific microflora in soil, while certain microbial species may take advantage of allelochemicals in soil [2]. Nitrogen derived from N-fixing leguminous *L. albus* is known to stimulate plant growth of many plant species [35] hence no inferences on possible stimulatory allelopathic effects would be appropriate, although stimulatory allelopathic effects have been reported [32].

4.4 *L. albus* L. v. Quilinock

The faster growth rate of *L. albus* L. v. Quilinock grown on Langgewens soil, which was stimulated by pot leachates from *H. vulgare, T. aestivum* or *L. multiflorum x L. perenne,* is congruent with findings on stimulation by grass species of plant growth [37]. Furthermore, PCA indicated that the effect of *L. multiflorum x L. perenne* on *L. albus* v. Quilinock was probably related to soil micro-organisms, which corresponds with results reported by [38] on the stimulation of crop growth by root exudates of certain weed species.

4.5 *L. multiflorum* v. Energa

The slower growth rate of *L. multiflorum* v. Energa grown on Langgewens soil, which resulted from *H. vulgare* pot leachate, confirms results by [39] and [40] who found that various plant species inhibited *L. multiflorum*. Results by [41] indicated barley leaves and roots to be the most phytotoxic parts reducing plant growth. However, the reported response varied depending on the source of allelochemical(s) (plant part) and the growth stage of the barley plant. PCA revealed that for Tygerhoek soil an association existed between soil micro-organisms and *L. multiflorum* v. Energa treated with *T. aestivum* or *L. multiflorum x perenne* pot leachates.

It was reported [42] that cotton seedling development was inhibited by aqueous extracts of wheat. Both positive and negative allelopathic effects by *Lolium rigidum* on *Lolium multiflorum* was reported by [43], while [44] reported inhibition of *L. rigidum* by *T. aestivum*.

4.6 *L. multiflorum x L. perenne*

L. multiflorum x L. perenne showed positive responses to Graminaceae species in that *T. aestivum* or *L. multiflorum x perenne* pot leachates stimulated its growth rate when grown on Langgewens soil. The significantly faster growth rate of *L. multiflorum x L. perenne* on Langgewens soil treated with *H. vulgare* pot leachates was revealed by PCA as a probable association with growth-promoting soil micro-organisms. In contrast, the non-significance observed for growth rate of this species on Tygerhoek soil, most probably indicates that either no growth-promoting or growth-inhibiting soil micro-organisms occurred, emphasizing the importance of location in plant-microbe interactions.

Generally, the investigated plant species showed not only different plant-micro-organism associations, thus confirming results by [45] and [2], but results also point to the presence of different allelochemicals for each plant type. Also, results showed [2] that soil microbial

populations were affected by the compounds released from allelopathic cultivars. Allelochemicals can be degraded through chemical, photolytic and most importantly, microbial processes [46].

It was suggested [2,47] that allelopathic crops and weeds could modify the microbial community structure in soil to their advantage through the release of allelochemicals. Own findings strengthen the significance of soil micro-organisms in chemical root exudates and allelochemical-mediated interactions between plants, whether to lessen or to magnify effects. It has been demonstrated that not only the originally exuded compounds but also their derivatives can have allelopathic activity [48].

Speculation [49] pointed to the secretion of allelopathic compounds into the rhizospere which may provide a competitive advantage for root establishment through local suppression of pathogenic soil micro-organisms and inhibition of the growth of competing plant species. Results by [50] suggested that rhizospere micro-organisms have positive or negative effects on plant growth and morphology by affecting the plant hormone balance, plant ensymatic activity, nutrient availability and toxicity, and competition with other plants. Plants can modify the rhizospere in other ways than through the release of allelochemicals, e.g. by causing changes in soil pH, nutrient and moisture levels and as a result can modify the local plant community. Allelochemicals released from crop plants may stimulate microbial biomass and populations in the rhizosphere [51] which in turn, may alter allelochemicals through microbial transformation [46].

Although this study did not clarify interactions between specific microbial populations and individual components of root exudates, it was evident that allelochemicals exuded from roots may influence soil microbial population and community structure. Thus, chemical interference from crop allelopathy in crop rotational systems may occur not only in weeds, but also in soil microbes and *vice versa.*

5. CONCLUSIONS

Crop cultivars and weeds may modify the soil micro-organism populations to their advantage and to the disadvantage of other species by the release of root exudates that apparently differ in composition between plant species. Findings indicate that root exudates can influence the microbial community structure in the rhizosphere and hence plant-microbial interactions of which the allelopathy phenomenon is likely part of. Differential plant growth and microbial responses observed on the two soils used in the study suggest that locality is an important factor governing plant-plant and plant-microbe interactions. Also, the possible positive or negative effects of micro-organisms present in a particular soil type on plant growth should be considered in allelopathy research. We contend that microbial community analysis with the Biolog EcoPlate™ could be used as an indicator of the allelopathic activity of crop or weed species.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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