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ORIGINAL RESEARCH ARTICLE

Root exudates from weedy ryegrass hybrid type and selected crop plants affect soil microbial communities in two soil types of the Western Cape, South Africa

Michael Ignatius Ferreira[1,*], Anélia Marais[1], Alfred Botha[2], Carl Frederick Reinhardt[3], Marieta van der Rijst[4]

[1] *Directorate Plant Sciences, Western Cape Department of Agriculture, Private Bag X1, Elsenburg 7607, South Africa*

[2] *Department of Microbiology, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa*

[3] *Unit for Environmental and Management Sciences, School of Geo- and Spatial Sciences, North-West University, Potchefstroom 2520, South Africa*

[4] *Agricultural Research Council, Biometry, Private Bag X5026, Stellenbosch 7599, South Africa*

*** Corresponding author:** Michael Ignatius Ferreira, Mike.Ferreira@westerncape.gov.za

ABSTRACT

All growing plant roots have the ability to produce root exudates to which soil microbes are attracted. The objective of this study was to utilise the Biolog EcoPlate™ system to indicate the impact of soil type on soil microbial communities in the rhizosphere following treatment with pot leachates that contain various plant root exudates. A greenhouse experiment was conducted in 2021 and repeated in 2022 from May until July (southern hemisphere) in this winter rainfall area with Mediterranean climatic conditions. This ensured that natural daylight hours in the greenhouse coincided with those experienced in the field by winter-growing crops, from seeding until maturity (May to October). Pot leachate that contained various plant root exudates from six donor plant species (wheat, barley, two lupine cultivars, ryegrass pasture type, and weedy ryegrass hybrid type) was utilised as treatment for respective recipient pots, in which wheat (*Triticum aestivum* v. SST 027) was grown as a test plant. Recipient plants were grown in two sets of pots, each with two different soil types. Soil samples from recipient pots were used to inoculate the Biolog EcoPlate™ system, and the carbon utilisation patterns obtained in this process were compared to the soil microbial populations present in the soil samples collected prior to treatments. Pot leachate treatment effects on the two soil types differed. Similarly, the treatments had differential effects on the measured soil microbial populations of the recipient wheat plants. Results indicate that the pattern of substrate utilisation by the Biolog EcoPlate™ methodology indicates changes in the number of colony forming units in the soil. In this regard, it was clear that ryegrass pasture variety and weedy ryegrass hybrid type caused similar effects on the soil bacteria communities in the rhizosphere. It is concluded that the primary impact of soil type is distinct microbial communities as an important factor regulating plant and plant-microbe synergy. Secondly, due to the strong selective forces root exudates have on the soil microbiome, conspicuous microbial communities in the rhizosphere of each plant species will continue to develop over time.

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Keywords: Biolog EcoPlate™ system; microbiome; pot leachate; rhizosphere; soil microbes

1. Introduction

All growing plant roots have the ability to secrete chemical compounds in response to biotic and abiotic influences[1,2]. These root exudates can comprise a wide variety of organic compounds, including carbohydrates, proteins, vitamins, and amino acids[2,3] and can even impact the genes playing roles in plant-microbe interaction[4]. Wang et al.[5] reported that root exudation is an active process, causing plants to alter the rhizospere actively for their own benefit. Consequently, plant root diffusions benefit the welfare of microbial communities[6].

Findings suggest that root exudates can also affect the microbial community structure in the rhizosphere and that the type as well as the rate thereof can be controlled by both plants and microbes[7,8]. Earlier, Feng et al.[9] reported that plants play an active role in the specific organisms that inhabit their root zone and that many of the microbes are adapted to the specific plants. In addition, by releasing root secretions that appear to differ in composition in different plant species, crop cultivars and weeds may alter soil microbial populations to their advantage and to the detriment of other species[7,10].

Furthermore, a gigantic and exceedingly divergent microbial community resides in close partnership with plants, either in the rhizosphere or within plant tissue[6]. The rhizosphere is a juncture for soil microbes[11], because the chemical exudates from plant roots are decisive cues for microbial communities as they can either entice or repel microbes from the plant[12]. These microbial communities may be favourable or disadvantageous to plant progress and growth, or they may have no noticeable outcome on plants whatsoever[6].

Roots serve as messengers between the plant and its associated microbes[2], while root secretions serve as an important carbon and energy source for microbes found in the rhizosphere[8] but are plainly difficult to isolate and define[13]. Some of the root exudates may also act as allelochemicals and mediate interactions between plants and plants[5], or plants and other organisms in the rhizosphere[5,10]. However, soil microbes can decrease or promote allelopathic responses once chemicals are secreted into soil[14].

Garland and Mills[15] introduced the Biolog™ methodology to visualise the physiological properties of microbial communities during both the characterisation of community functional properties and the assessment of community dynamics. In applied ecological research, the Biolog EcoPlate™ is used both to determine the stability of a normal population and to detect and evaluate changes (www.biolog.com). The Biolog EcoPlate™ system offers 31 different carbon sources for potential microbes in the soil solution. The utilisation of these carbon sources will be specific to a microbial community, which then provides the observer with a physiological profile of the microbial community under observation. Therefore, any changes in the composition of this microbial community as a whole will be reflected in changes in the pattern of carbon resource utilisation[16].

Although molecular methods are often used to measure changes in microbial communities[17] it has been found that the cultivable microbial fraction of the soil community often contributes most to the functionality of the ecosystem[18,19]. Using the Biolog EcoPlate™ system in conjunction with molecular methods, Moretti et al.[20] confirmed that the phylogenetic diversity appeared to be associated with the metabolic diversity of bacteria in a waste water treatment pond. Soil microbial community analyses have been performed by a number of researchers who have used the Biolog EcoPlate™ system with varying degrees of success[16,21–23] including allelopathy research[24–26]. Zhang et al.[21] used the Biolog EcoPlate™ methodology to differentiate between areas infected by *Solidago canadensis* and natural sites, while Grayston et al.[27] were able to associate distinct carbon use patterns with different plant species.

Since microbes are the pivotal operators for the indispensable onset of life, Schloter et al.[28] concluded that the influence of agricultural production practices on the soil microbiome must be appraised. Weedy ryegrass hybrid types, which grow in close proximity and strongly compete with crop plants for growth resources, should also have a prominent influence on the soil microbiome. Additionally, the rhizosphere serves as a carbon-rich habitat and the founding basis for microbial communities[29]. In the current study, plant root exudates from various crop and weedy ryegrass hybrid-type plants were utilised in this regard. Therefore, the objective of this study was to utilise the Biolog EcoPlate™ system with wheat as a test plant, in order to indicate the role of soil type in the impact of pot leachate on changes in soil microbial communities in the rhizosphere.

2. Materials and methods

To examine the microbial assortment in earthbound habitats as a basic and fundamental part of ecosystem activity[30], several methods are available for the analyses of pot leachate, but it was decided to uitilise the Biolog EcoPlate™ system since it is an inexpensive, suitably equipped, and swift technique for exploring the physiological heterogeneity in their surroundings[30]. It can also be relied upon to provide a wide range of information about microbial communities. In this study, it was utilised in order to determine changes in microbial populations over the trial period.

A greenhouse experiment was conducted in 2021 and repeated in 2022 from May until July (southern hemisphere) in this winter rainfall area with Mediterranean climatic conditions. This ensured that natural daylight hours in the greenhouse coincided with those experienced in the field by winter-growing crops, from seeding until maturity (May to October). Pot leachate was obtained from various donor plant species that were grown in pots under environmentally controlled conditions in a greenhouse. All pots contained unsterilised soil to simulate field conditions as closely as possible. This allowed for determining the extent to which the microbial communities of receiver pots planted to wheat only were structured by inherent soil properties in conjunction with factors from selected crops and weedy ryegrass hybrid-type root exudates.

2.1. Greenhouse pot experiment

Detection of possible changes in substrate utilisation (carbon sources) and monitoring the growth of heterotrophic organisms of fast-growing bacteria within 48 h were enabled by utilising the same soil sample to inoculate both nutrient agar plates[31] and the Biolog EcoPlate™ system. The six types of plants used in this greenhouse study that were utilised as leachate donors consisted of wheat (*T. aestivum* v. SST 027), barley (*H. vulgare* L. v. Clipper), lupine (*Lupinus albus* L. v. Tanjil and v. Quilinock), ryegrass pasture variety (*Lolium multiflorum* Lam. v. Energa), and weedy ryegrass hybrid type (*L. multiflorum* × *L. perenne*)[32]. Procedures in the greenhouse were based on the method followed by Ferreira et al.[25] and entailed two sets of pots that served as recipients (receivers) of the aforementioned donor leachate. Donor root leachate, comprising a wide variety of organic compounds[2], including soil microbes in the soil solution and allelopathic substances, was leached by surface irrigation and collected in brown glass containers as pot leachate for subsequent use. All recipient pots were planted with wheat (*T. aestivum* v. SST 027) as a test plant and were separately treated with all respective donor pot leachates.

Each set of recipient pots contained an unsterilised soil type collected 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 Department of Agriculture. Soils from Langgewens are residual (pH 6.3) and of Glenrosa (Entisol) type[33]. Tygerhoek soils are poorly developed residual soils (pH 5.2) and of Mispah (Entisol) type. After seeding in the greenhouse, plants were grown until termination at 77 days after planting. The greenhouse was set at a

constant temperature of 18 °C, and natural light was used, resulting in the plants being exposed to the normal day length for the crop growth period from May to October (southern hemisphere). The trial layout was a randomised block design with four replicates and was repeated once to obtain a comprehensive data set.

2.2. Soil microbial community analyses

Baseline soil microbial analyses that preceded the pot experiment were performed on both soil types to serve as reference points. After concluding all treatments, two bulked soil samples were again collected from each pot and analysed for comparative purposes. All the soil sampled in this way was added to 90 mL of sterile distilled water. After that, it was shaken by hand for 10 min and then allowed to settle for 2 h. Following settling, 100 µL aliquots of the supernatant were pipetted into the wells of the Biolog EcoPlate™ system (Biolog Inc., Haywood, California, USA) as a soil suspension and then incubated in the dark for 48 h at 22 °C. After this period, the 32 wells (each with a different carbon substrate plus one without substrate serving as a control) were assessed for colour development. The use of the carbon source in each well, indicated by a change of the tetrazolium dye, was then scored as 0 (carbon source not used) or 1 (carbon source used). The use of a carbon source (positive reaction) was indicated by a colour change compared to the control without any carbon source.

Heterotrophic, culturable microbes were determined in the soil samples. A 100-µL aliquot of each dilution in a soil dilution series (10^{-1}–10^{-5}) was transferred to a Petri® dish with nutrient agar[31] and spread over the surface with a sterile glass rod, after which it was incubated in the dark for five days at 22 °C. After five days, the number of colony-forming units in the 10^{-5} dilution was chosen on the basis that it could feasibly be counted. Following its counting, it was log-transformed to display the log of estimated colony-forming units (CFU) per gram of soil.

2.3. Statistical analysis

In order to simplify interpretation, the 31 carbon sources of the Biolog EcoPlate™ system were separated into six chemical groups (amino acids, amines, phosphorylated compounds, carboxylic acids, carbohydrates, and polymers), and the percentage utilisation of each group was calculated and used for statistical analyses. Since no year x treatment interaction was observed, data were averaged over years.

ANOVA was applied to the percentage utilisation of each of the six chemical groups to test for the main effect of locality and leachate treatments as well as any possible interactions[34]. Fisher's least significant difference was calculated to compare averages. Multivariate principal component analysis (MCA) was also applied to elucidate the association between localities, leachate treatments, and soil microbes[35]. Ward cluster analysis was applied within each locality to group treatments with similar utilisation patterns.

3. Results

Principal component analysis shows that the greatest contribution to the variation in the data was the differences between the soil types from the respective localities (**Figure 1**).

Bearing in mind that the experiment was carried out in a greenhouse under controlled conditions, analyses of prior microbial samples indicate intrinsic differences in the number of colony-forming units in both localities.

The first two axes of the principal component analysis explain 78.99% of the variation in the data (**Figure 1**). The first principal component (PC) F1 (61.85%) largely distinguishes localities based on carbon resource utilisation and indicates that Tygerhoek (on the right-hand side) is positively associated with PC F1, while most Langgewens treatments are positioned in the two quadrants on the opposite side. The higher carbon

resource utilisation at Tygerhoek is also reflected in **Table 1**, which shows the average utilisation of carbon sources per chemical group for soil from both localities. Following treatment with the leachate from the respective donor pots, significant differences were observed for all measured groups, except for amino acids and heterotrophic organisms. The second PC F2 (17.14%) largely associates with heterotrophic organisms and distinguishes lupine v. Quilinock from the respective localities (square cosine > 0.5). Overall, it seems that the soil from Langgewens supported more heterotrophic organisms and grouped them in the top and bottom quadrants on the left-hand side. In contrast, the individual groups of carbon sources used by microbial communities are mostly grouped in both quadrants on the right, indicating prominence in the Tygerhoek soil (**Figure 1**).

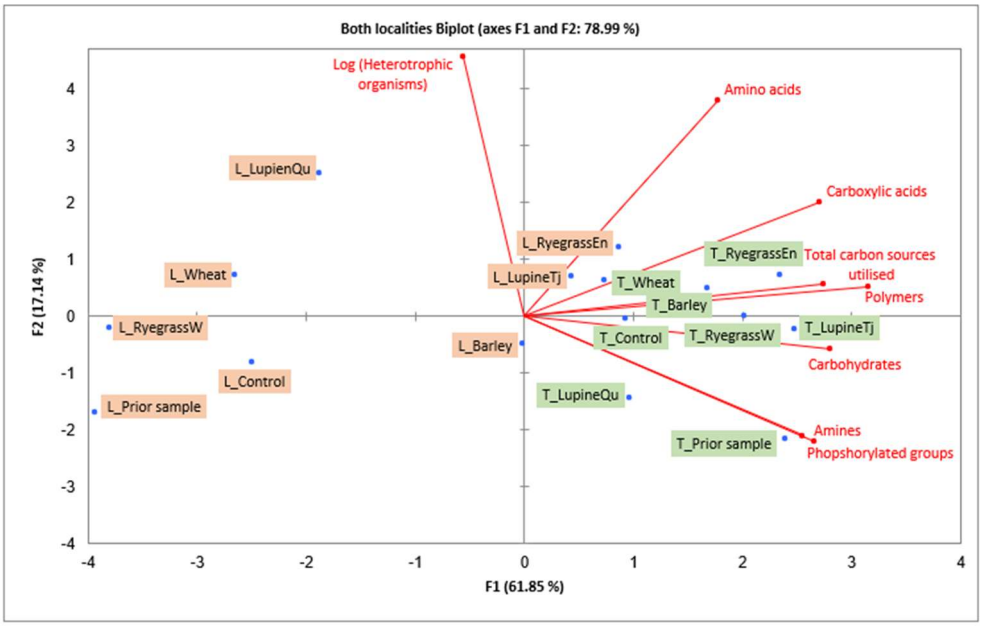
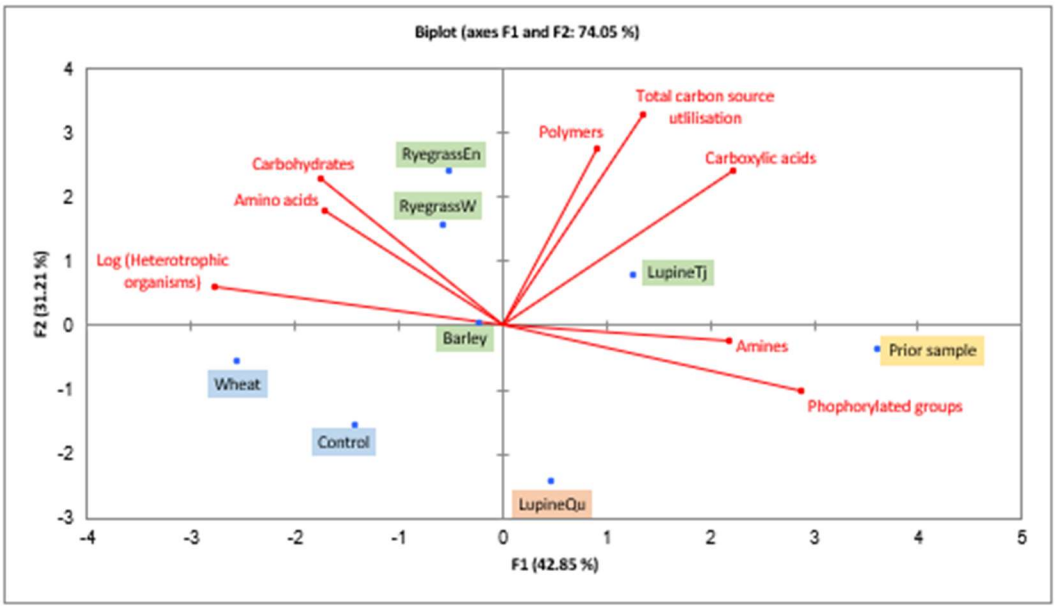


Figure 1. Principal component analysis of the average utilisation of carbon sources by six chemical groups in two soil types sampled from the respective localities, namely Langgewens and Tygerhoek. (L - Langgewens and T – Tygerhoek; LupineQu - lupine variety Quilinock; LupineTj - lupine variety Tanjil; RyegrassE - ryegrass pasture variety Energa; RyegrassW - weedy ryegrass hybrid type).

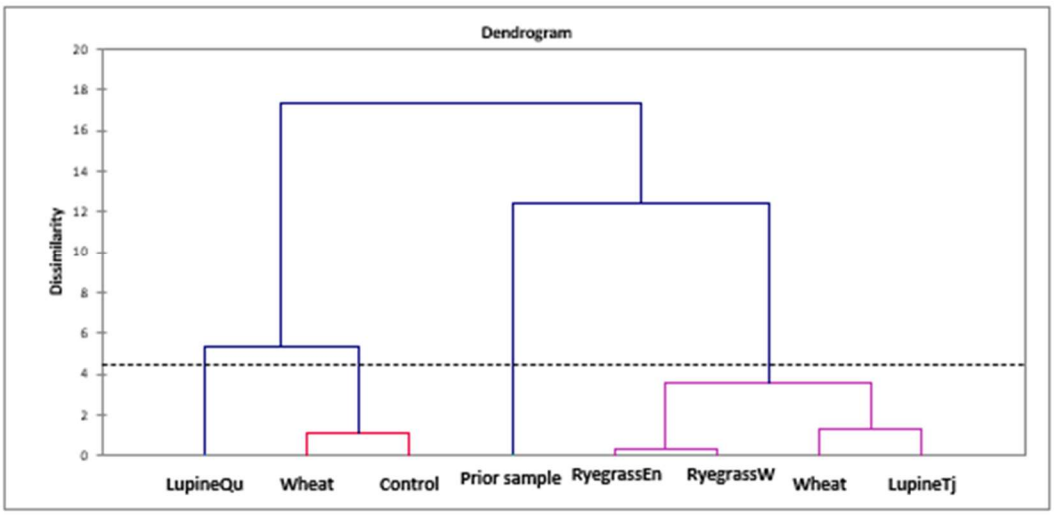
Table 1. Average utilisation of carbon sources by six chemical groups and log heterotrophic growth after leachate treatments of Tygerhoek and Langgewens soils.

Tygerhoek	**Log heterotrophic organisms**	**Average utilisation of carbon sources**					
Treatments		**Amines**	**Aminoacids**	**Carbo-hydrates**	**Polymers**	**Carboxylicacids**	**Phosphorylated groups**
Prior soil sample	7.3937^{a}	5.376^{ab}	6.452^{b}	16.129^{a}	0.750^{a}	0.667^{a}	6.452^{a}
Barley	8.2526^{a}	4.839^{ab}	11.290^{a}	16.129^{a}	0.625^{a}	0.611^{a}	3.226^{b}
Wheat	8.5792^{a}	1.613^{b}	9.667^{ab}	19.355^{a}	0.625^{a}	0.500^{a}	3.226^{b}
Lupine v. Tanjil	8.3314^{a}	6.452^{a}	9.667^{ab}	17.742^{a}	0.625^{a}	0.667^{a}	4.839^{ab}
Lupine v. Quilinock	8.0881^{a}	4.839^{ab}	6.452^{b}	16.129^{a}	0.625^{a}	0.500^{a}	4.839^{ab}
Ryegrass v. Energa	8.2041^{a}	3.226^{ab}	11.290^{a}	19.355^{a}	0.875^{a}	0.611^{a}	3.226^{b}
Weedy ryegrass hybrid type	8.5563^{a}	4.839^{ab}	8.065^{ab}	20.968^{a}	0.750^{a}	0.611^{a}	3.226^{b}
Control	8.4147^{a}	4.839^{ab}	9.667^{ab}	17.742^{a}	0.500^{a}	0.500^{a}	3.226^{b}
Langgewens	**Log heterotrophic organisms**	**Average utilisation of carbon sources**					
Treatments		**Amines**	**Aminoacids**	**Carbo-hydrates**	**Polymers**	**Carboxylicacids**	**Phosphorylated groups**
Prior soil sample	7.9543^{a}	1.075^{ab}	4.301^{a}	9.677^{a}	4.301^{a}	3.226^{b}	0.000^{a}
Barley	7.4647^{b}	3.226^{ab}	9.677^{a}	12.903^{a}	6.452^{a}	16.129^{ab}	1.613^{a}
Wheat	9.4163^{a}	1.613^{ab}	6.452^{a}	9.677^{a}	3.226^{a}	11.290^{ab}	1.613^{a}
Lupine v. Tanjil	8.3257^{a}	4.839^{a}	11.290^{a}	12.903^{a}	6.452^{a}	16.129^{ab}	1.613^{a}
Lupine v. Quilinock	9.3217^{a}	0.000^{b}	9.677^{a}	6.452^{a}	6.452^{a}	17.742^{ab}	0.000^{a}
Ryegrass v. Energa	8.4945^{a}	1.613^{ab}	9.677^{a}	16.129^{a}	9.677^{a}	19.355^{a}	1.613^{a}
Weedy ryegrass hybrid type	8.3920^{a}	0.000^{b}	6.452^{a}	6.452^{a}	4.839^{a}	6.452^{ab}	0.000^{a}
Control	7.4772^{a}	1.613^{ab}	8.065^{a}	9.677^{a}	3.226^{a}	11.290^{ab}	0.000^{a}

Based on carbon source utilisation patterns by the six chemical groups of the Biolog EcoPlate™ system (**Figure 2**), cluster analysis grouped the leachate treatments into four main groups for Tygerhoek soil. The prior soil sample separated from all other treatments, indicating that all treatments caused differences in the carbon source utilisation of soil microbes. Noteworthy is that ryegrass pasture variety and weedy ryegrass hybrid type grouped together in the top left quadrant, indicating that it caused similar effects on the soil bacteria in the rhizosphere of the recipient wheat plants.



(a)



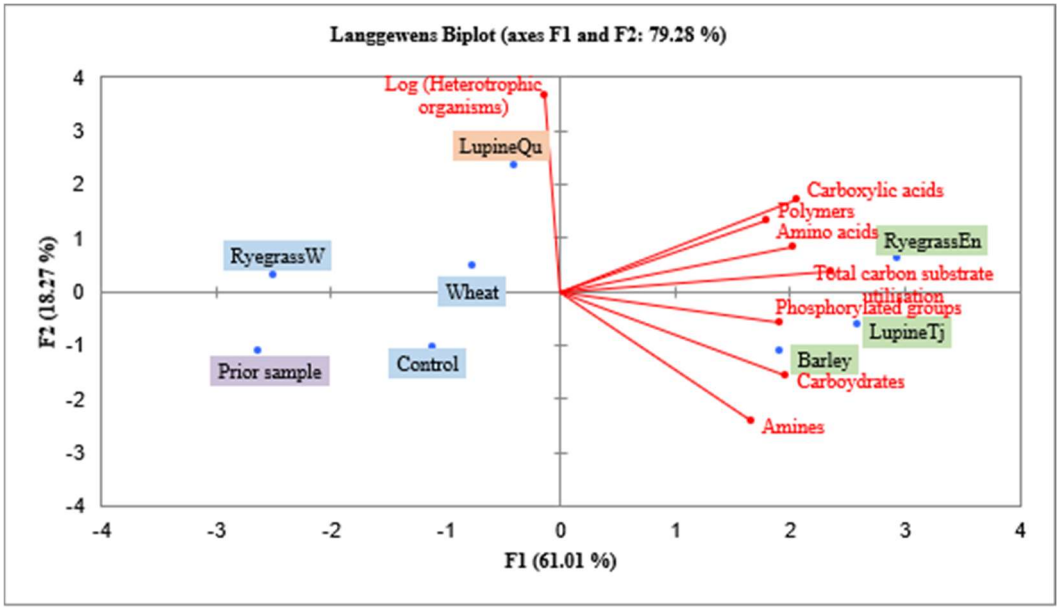
(b)

Figure 2. (a) Principal component analysis of Tygerhoek soil indicating the microbial analyses of prior soil samples and after it was subjected to pot leachate treatments. (LupineQu = lupine variety Quilinock; LupienTj = lupine variety Tanjil; ryegrassEn = ryegrass pasture variety Energa; ryegrassW = weedy ryegrass hybrid type; prior sample = soil sample preceding treatments); **(b)** Dendrogram of Tygerhoek soil indicating the microbial analyses of prior soil samples and after it was subjected to pot leachate treatments. (LupineQu = lupine variety Quilinock; LupienTj = lupine variety Tanjil; ryegrassEn = ryegrass pasture variety Energa; ryegrassW = weedy ryegrass hybrid type; prior sample = soil sample preceding treatments).

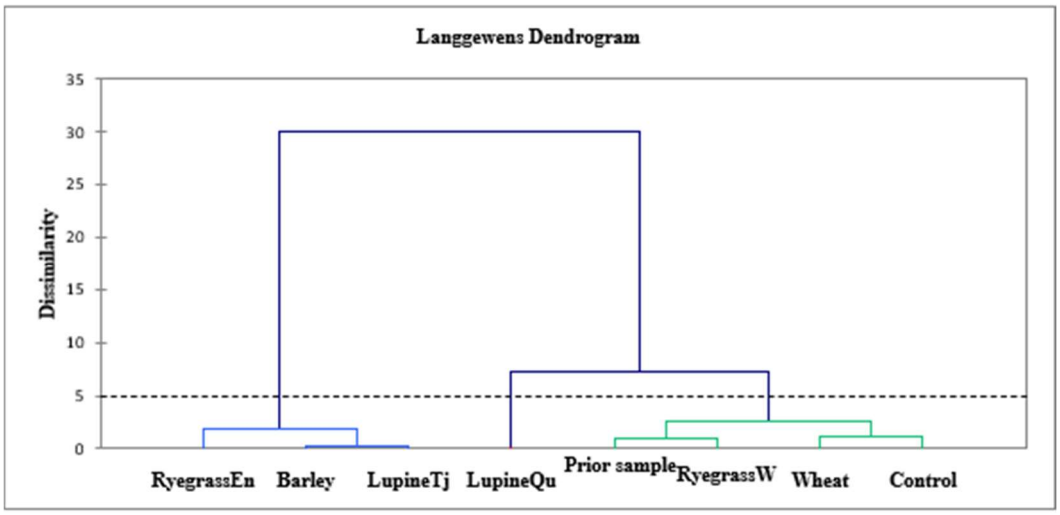
The first two axes (PC1 and PC2) of the principal component analysis at Tygerhoek explain 74.05% of the variation in the data [**Figure 2(a)**]. The first axis, F1 (42.85%), associates with positive utilisation of phosphorylated groups but fewer heterotrophic organisms (square cosine 0.77 and 0.83, respectively) and

separated mainly from the prior sample, wheat, and the control leachate treatments [**Figure 3(a)**] after it was subjected to treatment.

Even though only water was leached through the soil in the absence of plants, differences in the soil microbe community of the control were observed when compared to the soil sample taken prior to the commencement of the experiment. The second axis, F2 (28.27%), largely distinguishes lupine vs. Quilinock from the other leachate treatments based on the negative association with total utilisation of carbon sources. Evidently, the separate pattern of the prior soil sample shows that all treatments caused significant variation in the carbon source utilisation patterns of the soil [**Figure 3(b)**].



(a)



(b)

Figure 3. (a) Principal component analysis of Langgewens soil indicating the microbial analyses of prior soil samples and after it was subjected to pot leachate treatments. (LupineQu = lupine variety Quilinock; LupineTj = lupine variety Tanjil; RyegrassEn = ryegrass pasture variety Energa; RyegrassW = weedy ryegrass hybrid type; prior sample = soil sample preceding treatments); **(b)** Dendrogram of Langgewens soil indicating the microbial analyses of prior soil samples and after it was subjected to pot leachate treatments. (LupineQu = lupine variety Quilinock; LupineTj = lupine variety Tanjil; RyegrassEn = ryegrass pasture variety Energa; RyegrassW = weedy ryegrass hybrid type; prior sample = soil sample preceding treatments).

At Langgewens, cluster analysis separated leachate treatments into only three groups based on carbon utilisation from the six chemical groups of the Biolog EcoPlate™ system (**Figure 3**). Also, analyses showed that the control, wheat, and weedy ryegrass hybrid-type pot leachate treatments were all grouped together in the top left and bottom left quadrants. Leachate treatment of lupine v. Quilinock completely separated from other groups (top left quadrant), largely driven by higher numbers of heterotrophic organisms before treatment (square cosine = 0.71) but not associated with amine utilisation that is indicated in the bottom right quadrant [**Figure 3(a)** and **Table 1**].

The first two axes (PC1 and PC2) of the principal component analysis at Langgewens explain 79.28% of the variation in the data [**Figure 3(a)**]. The first axis, PC1 (61.01%), distinguishes ryegrass pasture variety, lupine v. Tanjil, and barley from other leachate treatments based on their positive association with the utilisation of carbon sources, as seen in **Figure 3(b)**. The second axis, PC2 (28.27%), largely associates with heterotrophic organisms and distinguishes lupine v. Quilinock from all other leachate treatments. Similarly, the dendogram [**Figure 3(b)**] shows that lupine v. Quilinock separated distinctly from all treatments.

Table 1 indicates that the measured soil organisms in the soil of the recipient wheat pots reacted differently to the respective leachate treatments. It is also evident from **Table 1** that the effect of the various treatments was not significant on the culturable heterotrophic organisms at Tygerhoek, although all treatments caused differences. However, the utilisation of various carbon sources was altered by the different leachate treatments when compared to analyses of the prior samples. At Tygerhoek, no treatments lead to significant differences in the use of amines by soil bacteria, although it was decreased by wheat leachate, while in contrast, it was increased by lupine v. Tanjil leachate. The utilisation of amino acids was significantly increased by the leachate of both barley and ryegrass v. Energa, while lupine v. Quilinock leachate apparently had no effect. The utilisation of the phosphorylated groups in Tygerhoek soil decreased, although the leachate effects of the two lupine cultivars were not significant. Even the control leachate, where only water was leached through the soil in the absence of plants, caused a significant decline in the utilisation of phosphorylated groups by the soil bacteria in the recipient wheat pots (**Table 1**).

Barley leachate significantly suppressed the growth of heterotrophic organisms in the Langgewens soil (**Table 1**). Although not significant, all other treatments stimulated heterotrophic organisms in this soil type. Furthermore, no treatment caused any significant differences in the utilisation of amines, carbohydrates, or polymers when compared to the analysis of the prior sample, although slight decreases and increases were observed.

Ryegrass var. Energa leachate was the only treatment that significantly increased the utilisation of carboxylic acids (**Table 1**). Apparently, no bacteria were able to consume phosphorylated groups in Langgewens soil, although the leachate of barley, wheat, lupine var. Tanjil, and ryegrass var. Energa stimulated the consumption of these phosphorylated groups, though not significantly so.

Means followed by the same letter are not significantly different at the 0.05 probability level, according to the Fisher Least Significant Differences test.

4. Discussion

In this study, we have shown that different soil types can have a major impact on the microbial community. Analyses of soil samples collected prior to conducting experiments provide evidence that the soil microbial populations at Tygerhoek are different from those at Langgewens. Contrasting responses of the microbes in the soil of the recipient wheat plants to the different leachate treatments, suggest that soil origin is an important factor controlling plant and plant-microbe interactions. This confirms findings by Xue et al.[36]

who reported that the soil type caused greater differences in microbial communities than the plants grown in the soil. Samuels et al.[37] elaborated on this and reported on the different ways microbes and geology are intertwined. Different microbes leave different weathering signatures in rocks and minerals, distinguishing a specific soil from soils of other origins. Microbes present in a particular soil type could potentially have negative or positive effects on vegetation[37].

Generally, the results of the present study demonstrated that the effects of two soil types on their microbial communities were disparate, irrespective of historical production systems or treatments with pot leachates that also included root exudates. This finding is consistent with that of Xue et al.[36] in that soil type primarily shapes microbial communities. In agreement with the present study and similar to results reported by Marais et al.[22], the mere presence of water that leached through the soil in the absence of plants caused differences in the soil microbe community when compared to the untreated soil sample. Furthermore, Xue et al.[36] showed that the assembly of microbial communities in agroecosystems responds both to factors outside the control of crop producers, such as soil type and climatic conditions, as well as to secondary factors under their control, including crop species, crop varieties, and crop rotational management.

Following pot leachate treatments, the control, wheat, and weedy ryegrass hybrid-type microbial communities were all assembled in the same PC (principal component) quadrants, as is evident by the analyses of the Langgewens soil type. Since wheat has been cultivated in this area for decades, coupled with the weedy ryegrass hybrid type as an important production constraint over this period, it is highly likely that an association of similar microbial communities evolved in this particular soil type. This is in agreement with Chen et al.[38] who reported that soil fungal and bacterial community composition can shift in response to agricultural management practices.

Furthermore, Brunel et al.[39] showed that carbon substrate utilisation patterns, as measured by the Biolog EcoPlate™ methodology, were mostly explained by vegetation type. Interestingly, following treatment of Tygerhoek soil in the present study, ryegrass pasture variety and weedy ryegrass hybrid type grouped together in the top left PC quadrant, indicating that it caused similar effects on the soil bacteria in the rhizosphere of the recipient wheat plants. This is consistent with Upton et al.[40] and Guo et al.[41] who demonstrated that, due to the strong selective forces crop plant communities have on the soil microbiome, distinct microbial communities in each production system will continue to develop over time. Oberan et al.[42] and Kong et al.[3] also reported particular plant species-microbe associations.

More specifically, the results of our study confirm that soil type, plant species, and variety, as well as microbes, each play a critical role in the formation of a specific soil microbial community. Earlier, Sasse et al.[32] highlighted the influence of plants on microbial communities. Consequently, some plants have microbial communities distinct from bulk soil, whereas other species have assembled a rhizobiome similar to bulk soil. The plant species included in crop rotations affect the microbial community through their intrinsic traits[43]. Furthermore, plant communities affect the architecture, heterogeneity, and dissemination of soil microbial communities[44]. The limited natural assortment of plant species is pointed out as an agent of changes in soil microbial functioning[45], with repercussions for the multiple processes of the soil microbiome[41]. In this regard, ryegrass pasture type was the only treatment in the current study that significantly increased the utilisation of carboxylic acids.

Although the Biolog EcoPlate™ methodology does not reveal the genetic composition of the bacterial community, it contributes invaluable comprehension of bacterial substrate use patterns and metabolic functional potential in their habitat[33]. As such, it could be applied as a monitoring method since it is an inexpensive, suitably equipped, reproducible, and easy technique to describe and compare bacterial

communities and their activity[33]. Contrastingly, Ge et al.[46] showed that the Biolog EcoPlate™ system is unable to evaluate the structure of microbial communities. Earlier, Stefanowicz[47] pointed out that only microbes that are cultivable and able to grow in high-nutrient conditions contribute to substrate utilisation.

Future research should involve studies aimed at fully understanding the functions and microbial requirements of a healthy crop, which would eventually lead to the directed design of customised microbial communities. Regarding weeds and their management, this might include studies on manipulating soil microbial communities to the point where it is utilised to be unfavourable and/or antagonistic for substrates of plant-associated microbes. This might involve uncovering an unhealthy rhizobiome that could be utilized to negatively affect the growth of a problematic weed in association with allelopathic root exudates. However, Li et al.[48] noted that soil microbes significantly decreased the allelopathic effects of leaf leachates. Also, the main allelochemicals of invader weeds were degraded more rapidly with increasing invasion history in the soil[48]. Korenblum et al.[49] emphasized that after the release of root exudates into the rhizosphere, the potency, scope, and biological actions of allelochemicals are explicitly shaped by microbes that can cause chemical modifications. Sasse et al.[32] believed this needed to be complimented with an enhanced comprehension of the substrate preferences of plant-associated microbes, their interactions, and the methods through which they benefit the plant. Sasse et al.[32] also suggested that profound future research is needed to reveal the precise mechanisms guiding plant-microbiome association and the possible beneficial functions of the microbial community. An increased aptitude for root morphology, exudation, and the transporters involved will likely enable the genetic engineering of plants with modified essential qualities to interact with specific beneficial microbes.

5. Conclusion

Evidence from this study showed that the pattern of substrate utilisation by the Biolog EcoPlate™ system indicates changes in the number of colony forming units in the soil. In this regard, it was clear that ryegrass pasture variety and weedy ryegrass hybrid type caused similar effects on the soil bacteria communities in the rhizosphere. Also, it is highly likely that an association of similar microbial communities evolved in the soil between wheat and weedy ryegrass hybrid types.

It can be concluded that soil type, plant species, and variety, as well as types of microbes, each play a critical role in the formation of a specific soil microbial community. The primary impact of soil type is distinct microbial communities as an important factor regulating plant and plant-microbe synergy. Secondly, due to the strong selective forces root exudates have on the soil microbiome, conspicuous microbial communities in the rhizosphere of each plant species will continue to develop over time.

Since the Biolog EcoPlate™ methodology is used both to determine the stability of a normal microbial population and to detect and evaluate community changes, it provides the observer with a physiological profile. However, it is not intended to reveal the genetic composition of the bacterial community, and we are unable to evaluate its structure, but it nevertheless contributes invaluable comprehension into bacterial substrate use patterns and metabolic functional potential in their habitat. As such, it could be applied as a monitoring method to describe and compare bacterial communities and their activity.

More work should be done on rhizospheric microbes and their interactions with one another and with plant hosts in order to pinpoint the specific microbial groups responsive to sustainable agricultural practices. This will inform agricultural producers about the technology and management decisions most likely to push their microbial communities toward that desirable state. In view of the global challenges of climate change and pollution of both air and water, more emphasis should be placed on manipulating soil microbial

communities to improve total yields and economic efficiency, which will reduce the environmental impacts of agriculture.

Author contributions

Conceptualization, MIF and AM; methodology, AB, AM and MIF; software, MVDR; validation MVDR and AM; formal analysis, MVDR and AM; investigation, AM and MIF; resources, AM and MIF; data curation: AM and MIF; writing-original draft preparation, AM and MIF; writing-review and editing, CFR; visualization, MIF; project administration, AM and MIF; funding acquisition, AM and MIF. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that they have no conflict of interest.

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