# RHIZOSPHERE MICROBIAL COMMUNITIES AND CARBON PARTITIONING UNDER ZERO-TANNIN LENTIL GENOTYPES

A Thesis

Submitted to the College of Graduate and Postdoctoral Studies

in Partial Fulfillment of the Requirements

for the Degree of Master of Science

in the Department of Soil Science

University of Saskatchewan

Saskatoon

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# ABSTRACT

The decomposition of soil organic carbon (C) is primarily mediated by soil microorganisms. By partitioning C through anabolic and catabolic processes, soil microorganisms control the flow of C through terrestrial ecosystems. As microorganisms metabolize organic compounds as a need to satisfy heterotrophic demands for C and energy, C partitioning should be related to both the physiology of the active microbial population and the biochemical quality of substrate. Zero-tannin (ZT) lentils have been selectively bred, for alterations in the phenylpropanoid pathway, to remove tannins from their seed coats. Any modification in a plant biochemical pathway has the potential to alter the tissue chemistry across the entire plant. The objective of this research was to examine soil microbial responses to ZT lentil genotypes both during plant growth and after, and to investigate how differences in the biochemical quality of aboveground (AG) and belowground (BG) plant tissues of ZT *versus* conventional tannin (TAN) genotypes affected soil C partitioning. Lentil plants were exposed to $^{13}CO_2$ during plant growth and harvested at flowering to coincide with peak rhizodeposition. Carbon isotope ratios in phospholipid fatty acids (PLFAs) of soil microorganisms revealed significant differences in microbial community structure and biomass between ZT and TAN genotypes. Further, microorganisms produced elevated levels of extracellular enzymes in the rhizosphere of ZT lentil genotypes. When AG and BG residues of each genotype harvested at maturity were incubated for a period of 107 days, microbial communities in microcosms incubated with BG residues produced proportionately more cell biomass per unit C degraded than microcosms incubated with AG residues. Further, unconstrained ordination by non-metric multidimensional scaling (NMDS) of Bray–Curtis dissimilarity revealed significant differences in decomposer community structure between AG and BG tissues but not between ZT and TAN genotypes. Extracellular enzyme activities were generally highest in control soils followed by BG soils, though no difference in enzyme activities were observed between genotypes. The results of this research suggest that biochemically complex compounds have the potential to be stabilized within the soil matrix via microbial residues. Moreover, differences in polyphenol content between TAN and ZT genotypes did not significantly affect respiration rates or cumulative C loss and may be an indication that C:N ratios are more important than the chemical composition of C compounds in regulating decomposition processes; while in the rhizosphere, small scale changes have the potential to alter soil process and C dynamics.

# ACKNOWLEDGEMNTS

I would like to express my most sincere appreciation to my supervisor Dr. Melissa Arcand for her guidance, inspiration, encouragement and continued support throughout this journey. Thank you for giving me the opportunity to grow as both an individual and as a student. Your mentorship is invaluable. I would also like to acknowledge the input and guidance from my advisory committee, Drs. Bobbi Helgason, Jeffrey Schoenau and Albert Vandenberg; as well as my external examiner Dr. Sylvie Quideau.

Financial support for this work was provided by National Sciences and Engineering Research Council of Canada (NSERC). Personal support was received from the Saskatchewan Pulse Crop Develop Board and the Department of Soil Science.

The completion of this undertaking could not be possible without the participation and assistance of many individuals of whose names may not all be enumerated though, their contributions are sincerely appreciated and gratefully acknowledged. Thank you Trang Phan, Erin White, Elizabeth Shirley, Jacob Eberley, Trianne Littlepine, Dale Barks and Maiah Tratch. I am especially grateful to Sarah Valentine for assisting me with the 5 a.m. labelling sessions in the greenhouse. Thank you Frank Krijnen for helping with all technical matters related to my $^{13}C$ enrichment chambers, Eldon Siemens for technical support in the greenhouse, Bobbi Helgason for advice and resources with the molecular work and Myles Stocki for the $^{13}C$ isotope analysis.

To my mom and dad, my masi and my brothers; I am extremely grateful for your unconditional love, patience and understanding. Your support has been monumental in the completion of this work.

Thank you to all of my friends for supporting me and sharing in all the excitements, achievements and frustrations along the way.

Lastly, I am incredibly thankful to Rozanne Poulson and Drs. Chantal Hamel and Xianzhou Nie for encouraging me to pursue graduate studies.

**PERSISTENCE**

**Per-sis-tence (noun) /pərˈsistəns/**
**Firm or obstinate continuance in a course of action in spite of difficulty or opposition.**

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| %CdfR | Percent carbon derived from roots |
| $^{11}C$ | Carbon-11, radioactive isotope of carbon |
| $^{13}C$ | Carbon-13, stable isotope of carbon |
| $^{14}C$ | Carbon-14, radioactive isotope of carbon |
| AG | Aboveground |
| AM | Arbuscular mycorrhizal |
| AMF | Arbuscular mycorrhizal fungi |
| ANOVA | Analysis of variance |
| Atom% | Atom percent |
| BG | Belowground |
| BGL | β-glucosidase |
| C | Carbon |
| C:N | Carbon to nitrogen ratio |
| C3 | Photosynthesis through Calvin cycle |
| C4 | Photosynthesis through Hatch-Slack pathway |
| CBH | Cellobiohydrolase |
| C-IRMS | Combustion-isotope ratio mass spectrometry |
| $CO_2$ | Carbon dioxide |
| CUE | Carbon use efficiency |
| cv | Cultivar |
| d | Day |
| DFR | Dihydroflavonol reductase |
| DNA | Deoxyribonucleic acid |
| DOC | Dissolved organic carbon |
| EOC | Extractable organic carbon |
| F3'5'H | Flavonoid-3’,5’-hydroxylase |
| FA | Fatty acid |
| FAME | Fatty acid methyl ester |
| g | Gram |
| GC-FID | Gas chromatography-flame ionization detection |

| | |
|---|---|
| GC-MS | Gas chromatography-mass spectrometry |
| GP:GN | Gram-negative to Gram-positive bacterial ratio |
| Gram- | Gram-negative bacteria |
| Gram+ | Gram-positive bacteria |
| h | Hour |
| HSD | Honestly significant difference |
| LC-MS | Liquid chromatography-mass spectrometry |
| L-DOPA | L-3,4-dihydroxy phenylalanine |
| MBC | Microbial biomass carbon |
| MEMS | Microbial efficiency-matrix stabilization |
| mg | Milligram |
| MUB | Methylumbelliferone |
| N | Nitrogen |
| NA | Natural abundance |
| ND | No data |
| NMDS | Non-metric multidimensional scaling |
| NMR | Nuclear magnetic resonance |
| P | Phosphorus |
| PERMANOVA | Permutational analysis of variance |
| PLFA | Phospholipid fatty acid |
| ppm | Parts per million |
| PPO | Polyphenol oxidase |
| qCO2 | Metabolic quotient |
| RNA | Ribonucleic acid |
| SIP | Stable isotope probing |
| SOC | Soil organic carbon |
| SOM | Soil organic matter |
| TAN | Genotype of lentil containing the functional tan gene |
| *tan* | Gene encoding tannin synthesis |
| VPDB | Vienna PeeDee Belemnite |
| WHC | Water holding capacity |
| ZT | Genotype of lentil containing a mutation in the tan gene |

# 1. GENERAL INTRODUCTION

## 1.1. Introduction

Soils represent a considerable stock of terrestrial organic carbon (C) with a storage capacity much larger than both the atmosphere and vegetation (Lal et al., 2015; Berhongaray et al., 2018). Therefore, depending on the balance between accumulation and decomposition of organic material, soils can act as either a potential sink or source for atmospheric $CO_2$. Soil C includes both inorganic C as carbonate minerals and organic C as soil organic matter (SOM), which is comprised of both living and non-living material in the soil. The living component is made up of plants, soil fauna and microbes while the non-living component includes a spectrum of materials from rhizodeposits, plant and animal residues at various stages of decomposition, cells and tissues of soil organisms, and substances synthesized by soil organisms (Paul et al., 2015). The majority of organic C results both directly from above (AG) and belowground (BG) plant structural components, rhizodeposits and leaf litter leachate, and indirectly by the transfer of C-enriched compounds from roots to soil microbes (Cotrufo et al., 2013). Therefore organic C is present in various forms differing in physical and chemical structure. Because soil organic C (SOC) is initially derived from photosynthetically captured C, plant productivity sets an absolute upper limit to potential C inputs to the soil system

Plant roots continuously produce and transfer C-rich compounds into the rhizosphere, generating a unique environment with a certain degree of specificity determined by several biotic and abiotic factors. The total C transferred from plant roots to the rhizosphere is referred to as rhizodeposition. Rhizodeposits consist of exudates, enzyme secretions, lysates from dead cells and mucilage (Grayston et al., 1996). The composition and rate of exudation depend on genetic factors and varies widely among plant species and developmental stage as well as environmental conditions (Kochian et al., 2005) such as nutrient availability, soil type, pH, temperature and moisture (Badri and Vivanco, 2009). The chemical composition of rhizodeposits is important in determining ecological function in the rhizosphere (Cheng and Gershenson, 2007).

As soil microorganisms are highly dependent on plants for substrate, rhizodeposits can mediate associations with specific microbes within the soil. For example, *Rhizobium* spp. are bacterial symbionts of legumes that are responsible for nitrogen (N) fixation, and communication between the two organisms is mediated through root-secreted flavones (Broeckling et al., 2008). Many chemicals present in root exudates have been found to act as substrates and chemotactic or signaling molecules to orchestrate changes in microbial composition (Badri and Vivanco, 2009) Badri et al. (2013) demonstrated that phenolic-related compounds modify the bacterial community by stimulating or inhibiting different community members. Therefore, the rhizosphere harbors an extremely complex microbial community that include saprotrophic and arbuscular mycorrhizal (AM) fungi, actinobacteria, Gram-positive (Gram+) and Gram-negative (Gram-) bacteria (Avis et al., 2008).

Microbial decomposition of organic matter is the metabolic degradation of plant, animal and microbial residues into simple organic and inorganic forms and plays a key role in global C cycling by directly linking terrestrial and atmospheric C pools (Houghton, 2007). Decomposition processes partition litter C by simultaneously returning $CO_2$ to the atmosphere through respiration, while generating other decomposition products that move through varying pathways of SOM formation (Cotrufo et al., 2015). Historically, differences in the decomposability of plant tissues were attributed to differences in their structural and chemical composition (Berg and McClaugherty, 2003; Rasse et al., 2005). Litter characterized by low N and high lignin, phenol and tannin content was generally described as recalcitrant, decomposing at lower rates and leaving relatively large amounts of residues in the soil (Melillo et al., 1982; Adair et al., 2008), hence contributing to the formation and persistence of SOM in the soil. Empirical evidence is building against the notion of intrinsic biochemical recalcitrance as the governing factor of SOM formation (Jenkinson et al., 1985; Kögel-Knabner et al., 2008; Marschner et al., 2008; Kleber and Johnson, 2010). It is now thought that stable SOM is largely independent of litter recalcitrance (Marschner et al., 2008) and products of microbial decomposition are likely to contribute more to stabilized SOM than original plant litter compounds (Mambelli et al., 2011). Moreover, labile plant inputs may be metabolized more efficiently than chemically complex compounds, resulting in a higher proportion of plant-derived C retained in soil as microbial residues (Cotrufo et al. 2013; Kallenbach et al. 2015). As microorganisms metabolize organic compounds as a need to satisfy

heterotrophic demands for C and energy (Frey et al., 2013), C partitioning should be related to both the physiology of the active microbial population and the biochemical quality of substrate.

Both substrate quality and microbial community composition are important factors governing microbial C use efficiency (CUE) (Manzoni et al., 2012; Sinsabaugh et al., 2013). Microbial CUE is the relative partitioning of substrate-C between catabolic respiration and anabolic processes that support growth, thus controlling the flow of C through terrestrial ecosystems. Microbial community composition can affect CUE as a result of varied life strategies (Fierer et al., 2007). For example, copiotrophic microorganisms thrive in nutrient rich environments and are characterized by high growth rates and fast turnover. By contrast, oligotrophs are characterized by their ability to grow under low substrate concentrations, and generally possess a higher substrate utilization efficiency (Fierer et al., 2007; Roller and Schmidt, 2015). Therefore, structurally different substrates can be processed by the same community with varying efficiencies.

Lentil (*Lens culinaris* Medikus) is an important grain legume crop that provides a significant source of protein, carbohydrates, and micronutrients for humans. Further, legumes are an important plant functional group since they can form a symbiosis with N-fixing *Rhizobium* and phosphorus-acquiring arbuscular mycorrhizal fungi (AMF). As atmospheric concentrations of greenhouse gases (GHGs) such as carbon dioxide, methane, and nitrous oxide are rapidly increasing, including pulse crops in rotations offer alternatives to systems relying exclusively on fertilization (Lemke et al., 2007). Polyphenols constitute a group of compounds derived from plant metabolism and are widely distributed in legume plants. Zero-tannin (ZT) lentils (Vaillancourt et al., 1986) have been selectively bred through alteration of the phenylpropanoid pathway to reduce tannins, a form of polyphenol, from their seed coats potentially altering the tissue chemistry and root exudate profile of the entire plant (Tilston et al., 2013; Bazghaleh et al., 2018). Any changes in plant derived C input has the potential to alter microbial community dynamics and biotic processing, thus shifting pulse-crop derived C trajectories through the soil.

### 1.2. Objective

The objective of this research was to examine soil microbial responses to ZT lentil genotypes both during plant growth and after, and to investigate how differences between the

biochemical quality of AG and BG plant tissues of ZT and traditional tannin (TAN) genotypes affected soil C partitioning. Lentil genotypes were exposed to $^{13}CO_2$ during plant growth and harvested at flowering to coincide with peak rhizodeposition. Carbon isotope ratios in phospholipid fatty acids (PLFAs) of soil microorganisms were used to determine the abundance and structure of microbial communities actively assimilating root derived C. Extracellular enzyme activities were evaluated to determine microbial function during plant growth and quantification of $^{13}C$ in the rhizosphere was used to evaluate relative C contributions to the soil. Aboveground and BG residues of each genotype harvested at maturity were incubated for a period of 107 days. Phospholipid fatty acids (FAs) were extracted to determine viable microbial biomass and community structure, extracellular enzymes were evaluated to examine microbial function and respiration rates were measured to determine microbial CUE in soils containing decomposing ZT and TAN lentil residues.

### 1.3. Organization of Thesis

The research presented in this thesis is organized in manuscript format. Following this introduction and the literature review presented in Chapter 2, two studies are presented in Chapters 3 and 4. These research chapters are organized following the C cycle, beginning with the input of C to soil via photosynthesis (Chapter 3) and concluding by examining the influence of ZT lentil genotypes on the potential for C loss from soils via microbial decomposition (Chapter 4).

The goal of the research presented in Chapter 3 was to trace photosynthetically fixed C from ZT lentil genotypes into the rhizosphere and to determine the short-term fate of microbially-processed C. A labeling system was designed to provide pulses of $^{13}C$-$CO_2$ to lentil plants grown under controlled conditions in a greenhouse. Carbon isotope ratios were used to calculate percent carbon derived from rhizodeposition (CdfR) as well as the portion of rhizosphere microorganisms actively assimilating root derived C.

In Chapter 4, soil microcosms with AG and BG residues originating from ZT and TAN lentil genotypes to assess the effect of residue biochemical quality on decomposition C dynamics. Phospholipid FA's were extracted to determine microbial abundance and community structure, extracellular enzymes were evaluated to examine microbial function and respiration rates were

measured to determine microbial carbon use efficiency in soils containing decomposing lentil residues.

Finally, Chapter 5 synthesizes major findings of the individual research studies. It includes conclusions of the thesis as a whole and suggests recommendations for future work. Literature cited within the thesis are compiled in the Reference section that follows Chapter 5.

# 2. LITERATURE REVIEW

## 2.1. The Carbon Cycle and Soil Carbon Inputs

Soil organic matter is the largest carbon (C) stock in terrestrial ecosystems, containing three times the amount of C than the atmosphere and four times the amount stored in all living plants and animals (Lal et al., 2015; Berhongaray et al., 2018); thus representing a potential sink for anthropogenic emissions of $CO_2$. Carbon is present as both organic and inorganic forms in the soil. Inorganic C exists in mineral forms, either from weathering of parent material or from the reaction of soil minerals with atmospheric $CO_2$ (Lal, 2006). The majority of soil organic carbon (SOC) enters the soil as a result of photosynthesis, the fixation of $CO_2$ into plant metabolic and structural tissues and is present in various forms differing in physical and chemical structure. For example, lignin, hemicellulose and cellulose are present in the structural components of above (AG) and belowground (BG) plant tissues and exist as polysaccharides in the soil while long chain *n*-alkanes originating from the epicuticular wax layers from leaves and roots comprise the majority of free lipids in soils (Dungait et al., 2012). Plants also invest a significant portion of their photosynthetically fixed C sources into the release of phenolic compounds, carbohydrates, sugars, amino acids and organic acids from their roots into the surrounding soil (Jones, 1998; Bais et al., 2006; Lareen et al., 2016). Over the life of a plant, 20–60% of C fixed during photosynthesis can be translocated belowground (Grayston et al., 1996; Kuzyakov and Domanski, 2000). The transfer of C involves multiple processes including root growth and maintenance, exudation and necrosis (Luster et al., 2009). Recently, it has been shown that the transfer of C-enriched compounds to the rhizosphere contributes a relatively large amount of C to the mineral soil (Sokol and Bradford, 2019) and that this contribution primarily consists of microbial compounds produced during degradation (Cotrufo et al., 2013).

## 2.2. The Rhizosphere Effect

The rhizosphere is the site of interface between soil, plant roots and soil microorganisms which all influence the environment and processes within (Lynch 1990). The term rhizosphere was first introduced by German scientist Lorenz Hiltner in 1904 to describe the soil zone surrounding

legume roots with intensive bacterial activity. Recently, this term was broadened to describe the soil zone immediately adjacent to plant roots and influenced by root activities (Pinton et al., 2001). During plant growth a variety of organic compounds are released from the roots through exudation, secretion and deposition making the rhizosphere an environment rich in nutrients and chemically very different than that of the bulk soil (Bais et al., 2006). The term rhizodeposition was first defined by Whipps and Lynch (1985) as ‘‘the material lost from plant roots, including water-soluble exudates, secretions of insoluble materials, lysates, dead fine roots and gases like $CO_2$ and ethylene’’. Rhizodeposits can be classified into two groups based on their utilization as microbial substrates. Low molecular weight organic compounds such as sugars, amino acids, organic acids, organic anions, phenolic compounds and various other secondary metabolites are readily assimilated by soil microorganisms; and high molecular weight organic exudates such as polysaccharides, proteins, pigments and mucilage require extracellular enzyme activity to break them down prior to assimilation (Meharg, 1994). Additionally, inorganic compounds such as inorganic ions, H+, water and electrons are also released by plant roots into rhizosphere soils (Bertin et al., 2003). The stimulation of soil microbial populations in the rhizosphere owing to the release of organic compounds, is termed the rhizosphere effect and has been recognized for decades (Lynch 1987).

The composition and rate of exudation depend on genetic factors and varies widely among plant species and developmental stage as well as environmental conditions (Kochian et al., 2005). Nutrition plays a role in root exudation (Badri and Vivanco, 2009). An in vitro study demonstrated that different growth media alters the composition of root exudates of a particular plant species. Further, limited nutrient availability increased the exudation of certain metabolites (Jones, 1998). Additionally, phosphorus deficiency results in enhanced root secretion of phenolic compounds in certain legume species, and the specificity of secretion varies with plant species (Dinkelaker et al., 1995; Chishaki and Horiguchi, 1997; Neumann and Römheld, 1999). Temperature, light and soil moisture also regulate root exudation. For example, the release of tannins and phenolic compounds in *Vicia faba* was significantly reduced at 4 °C compared to 30 °C (Bekkara et al., 1998). Light intensity alters the exudation of secondary metabolites owing to changes in photosynthesis (Watt and Evans, 1999). Accordingly, *Almus glutinosa* (L.) root exudates have increased flavonoid content under light conditions (Hughes et al., 1999). High soil moisture reduces oxygen availability in the soil, causing a shift from aerobic to anaerobic respiration, resulting in lowered

soil pH levels. To overcome the phytotoxicity associated with anoxia, plants release lactic acid into the rhizosphere (Xia and Roberts, 1994). The presence or absence of particular minerals and toxic metals in the soil can also alter the composition of root exudates. Plant roots secrete citric, oxalic and malic acids to detoxify aluminum in the soil (Badri and Vivanco, 2009) and the pattern of secretion varies with plant species (Ma, 2000; Liao et al., 2006; Wang et al., 2006). Exudation rates vary with plant developmental stage and within genotypes of a single species (Badri and Vivanco, 2009). Root exudation is positively correlated with root growth (García et al., 2001). That is, seedlings produce the lowest amount of root exudates which gradually increase and peak at flowering (Aulakh et al., 2001). Genotypes of A. thaliana were found to differ in the levels of malate present in their in exudates (Hoekenga et al., 2003). Similarly, qualitative and quantitative differences were observed in root exudation profiles between plant species and among genotypes of the same species (Cieslinski et al., 1997). Chemical and physical molecules that stimulate defense related or stress-induced responses are termed elicitors. Elicitors have been shown to induce roots to secrete phytochemicals in higher quantities than non-elicited plants (Gleba et al., 1999). Further, roots of elicited plants exude compounds not detected in the exudates of non-elicited plants. Moreover, these induced compounds vary between plant species (Badri and Vivanco, 2009). The secretion of phytochemicals and proteins from roots is an important way for plants to respond to and modify their environment, providing an important and unique habitat for soil microorganisms who play a significant role in carbon sequestration, decomposition and nutrient cycling which are vital processes for ecosystem functioning (Huang et al., 2014).

### 2.3. $^{13}$C Stable Isotope Probing of Microbial Compounds

The total C transferred from plant roots to the rhizosphere is referred to as rhizodeposition and is expressed in terms of C released by roots (CdfR). Identifying and quantifying rhizodeposition is highly challenging due to the diverse chemical nature of the organic compounds released. Recent advancements in analytical techniques such as gas chromatography-mass spectrometry (GC-MS) or GC-MS coupled with other techniques such as nuclear magnetic resonance (NMR) have been successfully used for the identification of the entire diversity of compounds released by roots, i.e. metabolomic fingerprinting (Oburger and Jones, 2018)

Isotopic tracing studies offer insight into the flow of fixed C (Nguyen, 2003). Rather than the identifying the entire suite of compounds released by roots, isotopic tracer studies allow us to

quantitatively trace the relative partitioning of freshly assimilated C within the plant-soil-microbe system (Nguyen, 2003; Pausch and Kuzyakov, 2018). Typically, tracer studies involve either pulse labelling of radioactive ($^{14}C$ and $^{11}C$) or stable isotopes ($^{13}C$), continuous labelling of the $^{13}C$ stable isotope or the difference of $^{13}C$ natural abundance between C3 and C4 plants (Kuzyakov and Domanski, 2000). In pulse-labelling studies, plants are exposed to labelled $CO_2$ for only a short period of time, providing information on C distribution at a specific plant developmental stage. As C allocation patterns change with plant development stage, a series of repeated pulse labeling events are necessary to obtain a reasonable estimate of below-ground C input (Qiao et al., 2014; Remus and Augustin, 2016; Yu et al., 2017; Austin et al., 2017; Sun et al., 2018) and still may not represent the distribution of total C (Kuzyakov and Domanski, 2000). In contrast, continuous labelling quantifies the total amount of assimilated C during the entire labeling period, thus providing the most accurate estimations of plant derived C contributions to the rhizosphere (Kochian et al., 2005; Esperschütz et al., 2009; Pausch et al., 2013). However, continuous labelling is costly and consequently, the number of studies using this approach is limited. Pulse- and continuous labelling experiments are frequently used to identify soil microbial populations actively involved in the processing of freshly deposited C (Hannula et al., 2017; Ahmed et al., 2018).

Stable isotope probing (SIP) of microbial compounds (PLFAs, DNA, RNA, proteins) enables us to link soil biogeochemical processes with soil microorganisms (Boschker and Middelburg, 2002; Evershed et al., 2006; Neufeld et al., 2007; Seifert et al., 2012). Phospholipid FAs are an essential structural component of all microbial cellular membranes and are distinct from those of plant and animal tissues. Additionally, some microbial FAs are specific to single microbial groups (Zelles, 1999). As a result, microbial lipids can be used as biomarkers to observe broad scale changes in the active microbial population of soil environments. Coupled with SIP using $^{13}CO_2$, it is possible to label microbial PLFAs and monitor the flow of photosynthetically fixed C into the portion of the microbial community actively assimilating root derived carbon. For example, Treonis et al. (2004) used SIP-PLFA to identify microbial groups actively involved in assimilation of root-derived C in limed grassland soils and found that Gram- bacteria are primarily involved in the rapid processing of fresh C input. Similarly, $^{13}C$ signatures in microbial PLFAs from a $^{13}C$ pulse-labelled field site in Germany showed that under elevated atmospheric $CO_2$ conditions, new rhizodeposited C is rapidly processed by fungal communities and only much later by the bacterial communities. In a laboratory incubation study using $^{13}C$-labeled ryegrass straw,

McMahon et al. (2005) showed differing C assimilation patterns in microbial PLFAs between leached and unleached straw. Williams et al., (2007) followed $^{13}C$ from field-labeled ryegrass and crimson clover residues into soil microbial PLFAs and showed that the relative contributions of residue C and soil C to specific PLFAs varied significantly with residue type. In Japan, PLFA studies have shown remarked differences in the dynamics of microbial communities associated with different rice straw parts (sheaths and blades) varying in C:N ratios (Nakamura et al., 2003; Sugano et al., 2005). Characterizing microbial community composition using PLFA-SIP may be useful in identifying differences in the microbial processing and fate of crop residue-derived C with contrasting biochemical quality.

### 2.4. Microbial Decomposition of Crop Residues

Microbial decomposition of organic matter is the metabolic degradation of plant, animal and microbial residues into simple organic and inorganic forms. Decomposition processes partition C by simultaneously returning $CO_2$ to the atmosphere through respiration, while generating decomposition products that can either be occluded within soil aggregates (Dungait et al., 2012) or become associated with silt and clay sized minerals in the soil (Grandy and Neff, 2008; Cotrufo et al., 2013). The organic matter added to soil, both from AG and BG detritus or through rhizodeposition is one of the energy (C) and nutrient sources (N, P) required for soil microorganisms (Kogel-Knabner, 2017). In agricultural soils, it is one of the main sources of C but not of nutrients.

Crop residues differ in their structural and chemical composition (Adair et al., 2008; Aber and Melillo 1982). Residue decomposition rates are generally negatively related to the amount of recalcitrant compounds such as lignin and polyphenols, present in their biomass (Bertrand et al., 2006; Castellano et al., 2015; Aber and Melillo, 1982). Aboveground crop residues are considered high quality in comparison to BG residues, which are relatively recalcitrant to decomposition (Bertrand et al. 2006; Rasse et al. 2005). Historically, differences in residue decomposition rates were correlated with the amount of stable C retained in the soil. Recalcitrant residues decompose at lower rates and were thus thought to leave relatively large amounts of remaining residue in the soil (Adair et al., 2008; Melillo et al., 1982). However, evidence is now building against the notion of intrinsic biochemical recalcitrance as the governing factor of soil organic matter (SOM) formation (Jenkinson et al., 1985; Kögel-Knabner et al., 2008; Marschner et al., 2008; Kleber and

Johnson, 2010). It is now thought that stable SOM is largely independent of litter recalcitrance (Marschner et al., 2008) and products of microbial decomposition are likely to contribute more to stabilized SOM than original plant litter compounds (Mambelli et al., 2011).

Although the biochemical composition of crop residues may not be seen in terms of its inherent recalcitrance, substrate quality influences SOM formation and stabilization through its effect on microbial processing. Microorganisms control two critical pathways in the formation of SOM. In the *ex vivo* modification pathway, C compounds are transformed without assimilation by soil microorganisms. Instead, microbial extracellular enzymes are synthesized to break down organic materials (Liang et al., 2017). The differences by which organic materials are modified, depend on plant and tissue type (Cornwell et al., 2008) and the active microbial population. For example, microorganisms in forest ecosystems are better adapted to degrading complex C compounds (Waldrop and Firestone, 2004), while grassland microorganisms degrade grass litter more efficiently than forest litter (Strickland et al., 2009). Evidence suggests that fungi preferentially degrade fresh plant litter due to their ability to synthesize a more potent suite of extracellular enzymes (Poll et al., 2006). Following enzymatic attack, C compounds can be directly sorbed to the mineral surface or occluded within aggregates. In the *in vivo* turnover pathway, microorganisms assimilate C for growth and metabolic function, thus leading to the deposition of microbially derived C to SOM (Liang et al., 2017). *In vivo* turnover is dependent on how microbial populations grow and assimilate organic substrate, thus changes in microbial anabolic capacity directly affect microbial contributions to SOM. For example, in forest ecosystems, bacterial biomass contributes more to SOM in broadleaf than coniferous systems, where fungal biomass is more prevalent (Liang et al., 2007). The consequences of both the *in vivo* and *ex vivo* pathways are thus primarily dependent on the composition and physiology of the microbial community as well as on substrate quality.

Both substrate quality and microbial community composition are important factors governing microbial C use efficiency (CUE) (Manzoni et al., 2012; Sinsabaugh et al., 2013). Microbial CUE is the relative partitioning of substrate-C between catabolic respiration and anabolic processes that support growth, thus controlling the flow of C through terrestrial ecosystems. Microbial community composition can affect CUE as a result of varied life strategies (Fierer et al., 2007). For example, copiotrophic microorganisms are characterized by high growth

rates and fast turnover and thrive in nutrient rich environments. By contrast, oligotrophs are characterized by slow growth, low rates of metabolism, and generally low population density, allowing them to grow under low substrate concentrations. Oligotrophs generally possess a higher substrate utilization efficiency (Fierer et al., 2007; Roller and Schmidt, 2015). Therefore, structurally different substrates can be processed by the same community with varying efficiencies.

Both AG and BG plant tissues are recognized as major contributors to SOM (Cotrufo et al., 2013), though there are contrasting accounts surrounding the primary source of C to the mineral soil. Some studies have shown the primacy of roots in forming the stable soil C pool (Austin et al., 2017; Sokol and Bradford, 2019). However, root detritus and exudates could not be partitioned in these field studies; thus not explicitly explaining the role of root detritus in the formation of SOM. Sokol et al. (2018) describe that it is the location and accessibility of C to microorganisms that determines the fate of C in the soil, though still not directly comparing AG and BG tissues in decomposition. Further, studies have shown that BG residues are respired at lower rates than AG residues (Silver and Miya, 2001; Wang et al., 2010; Birouste et al., 2012), yet this information is based solely on mass loss kinetics and we still lack knowledge based on mechanisms controlling the decomposition of AG and BG detritus. Other work has shown AG inputs significantly contribute to the stable soil C pool (Michalzik et al., 2003; Kalbitz and Kaiser, 2008), thus further confounding our understanding of the relative importance of AG and BG residues in the formation of stable SOM. These studies have left a gap in the current understanding of the role of AG and BG plant residues in the formation of stable SOM. We are still left wondering, to what extent are differences in AG and BG contributions to the stable C pool driven by differences in their biochemical quality?

### 2.5. Polyphenols and Zero-Tannin Lentils

Phenolic compounds are characterized by the presence of at least one hydroxyl group and an aromatic ring. They include phenolic acids, stilbenes, and flavonoids such as flavanones, flavones, dihydroflavonols, flavonols, flavan-3-ols, anthocyanidins, and proanthocyanidins (Vermerris and Nicholson, 2006) and are produced as a result of plant secondary metabolism. Studies have shown that phenolic acids significantly decrease the functional and genetic diversity of the soil microbial community (Wu et al., 2008) by selectively increasing the species of certain soil microbial species (Qu and Wang, 2008). Specifically, studies have demonstrated effects of

phenolic acids, such as ferulic acid (Zhou et al.,), vanillin (Zhou et al., 2018a), and *p*-coumaric acid (Zhou et al., 2018b), on specific soil microorganisms. Additionally, specific soil microorganisms use phenolic compounds as substrates or signaling molecules (Badri et al., 2013). However, many phenolic compounds have been shown to act as antimicrobials against plant soil-borne pathogens, such as *Fusarium graminearum* (Lanoue et al., 2010) and *Sclerotium rolfsii* (Sarma and Singh, 2003), as well as microorganisms with plant growth-promoting abilities, such as *Glomus intraradices* (Medina et al., 2011) and *Bacillus pumilus* (Wu et al., 2016). Flavonoids are a diverse class of polyphenolic compounds that are known to play multifunctional roles in both signal initiation of the legume-rhizobia symbiosis and in the establishment of arbuscular mycorrhizal symbiosis.

Tannins represent a complex range of phenolic compounds that are present in many plant tissues and as such, have diverse physiological and ecological functions (Xie and Dixon, 2005). For example, tannins present in the leaves of many plants provide a means of preventing insect herbivory, while tannins in the seed coats are suggested to function against fungal infection (Caldas and Blair, 2009). Moreover, condensed tannins also comprise a large group of plant phenolic secondary metabolites which have been reported to alter rhizosphere soil microbial communities (Tilston et al., 2013), inhibit microbial enzyme activities important for nutrient cycling (Appel, 1993), reduce rates of N mineralization thereby inhibiting nitrification (Hättenschwiler and Vitousek, 2000) and alter decomposition of crop residues (Liebeke et al., 2015).

In plants, phenolic compounds are synthesized through the phenylpropanoid pathway (Fig. 2.1, Lattanzio, 2013) and vary in their chemical structure (Fig. 2.2). A number of transcription factors that influence gene expression in this pathway have been identified (Mirali et al., 2016). Molecular analysis has demonstrated that the *tan* gene encodes a transcription factor that interacts with the regulatory genes in the biochemical pathway of phenolic compounds starting from flavonoid-3',5'-hydroxylase (*F3'5'H*) and dihydroflavonol reductase (*DFR*, Mirali et al., 2016). The *tan* gene influences pigmentation of stems and flowers. Non-mutant lentil plants have reddish stems, purple veins on floral tissues, and thicker, pigmented seed coats (Vaillancourt et al., 1986), while mutant genotypes are characterized by green stems and white flowers.

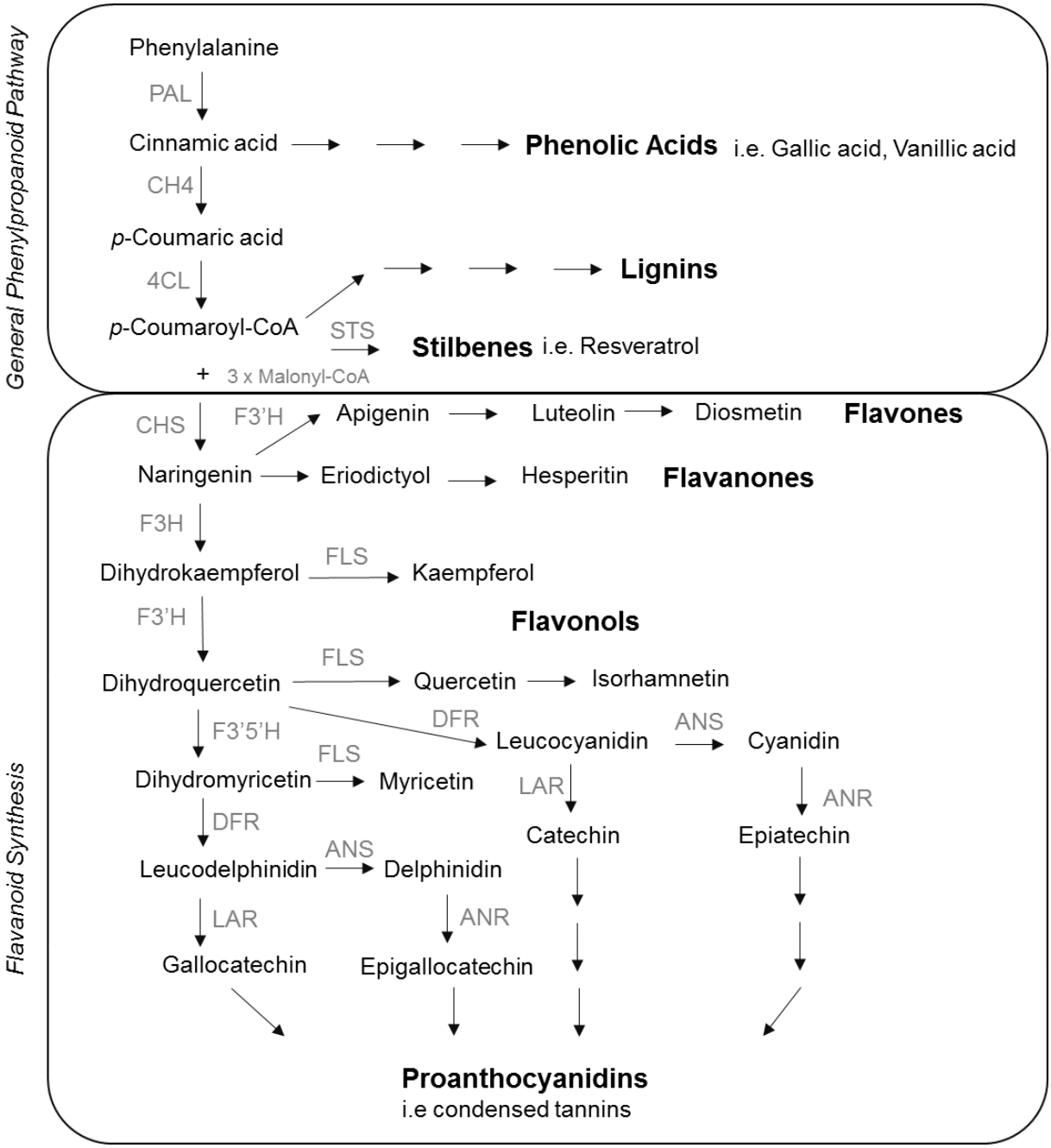


**Fig. 2.1. Simplified diagram of plant polyphenol synthesis through the phenylpropanoid metabolic pathway. Abbreviations: PAL, phenylalanine ammonia lyase; C4H, cinnamic acid 4-hydroxylase; 4CL, 4-coumaric acid:CoA ligase; STS, stilbene synthase; CHS, chalcone synthase; F3H, flavanone-3-hydroxylase; F3'H, flavonoid-3'-hydroxylase; F3'5'H, flavonoid-3',5'-hydroxylase; FLS, flavonol synthase; DFR, dihydroflavonol reductase; ANS, anthocyanidin synthase; LAR, leucoanthocyanidin reductase; ANR, anthocyanidin reductase; gluc, glucoside. Modified from Mirali et al. (2016) and Durand-Hulak et al. (2015).**

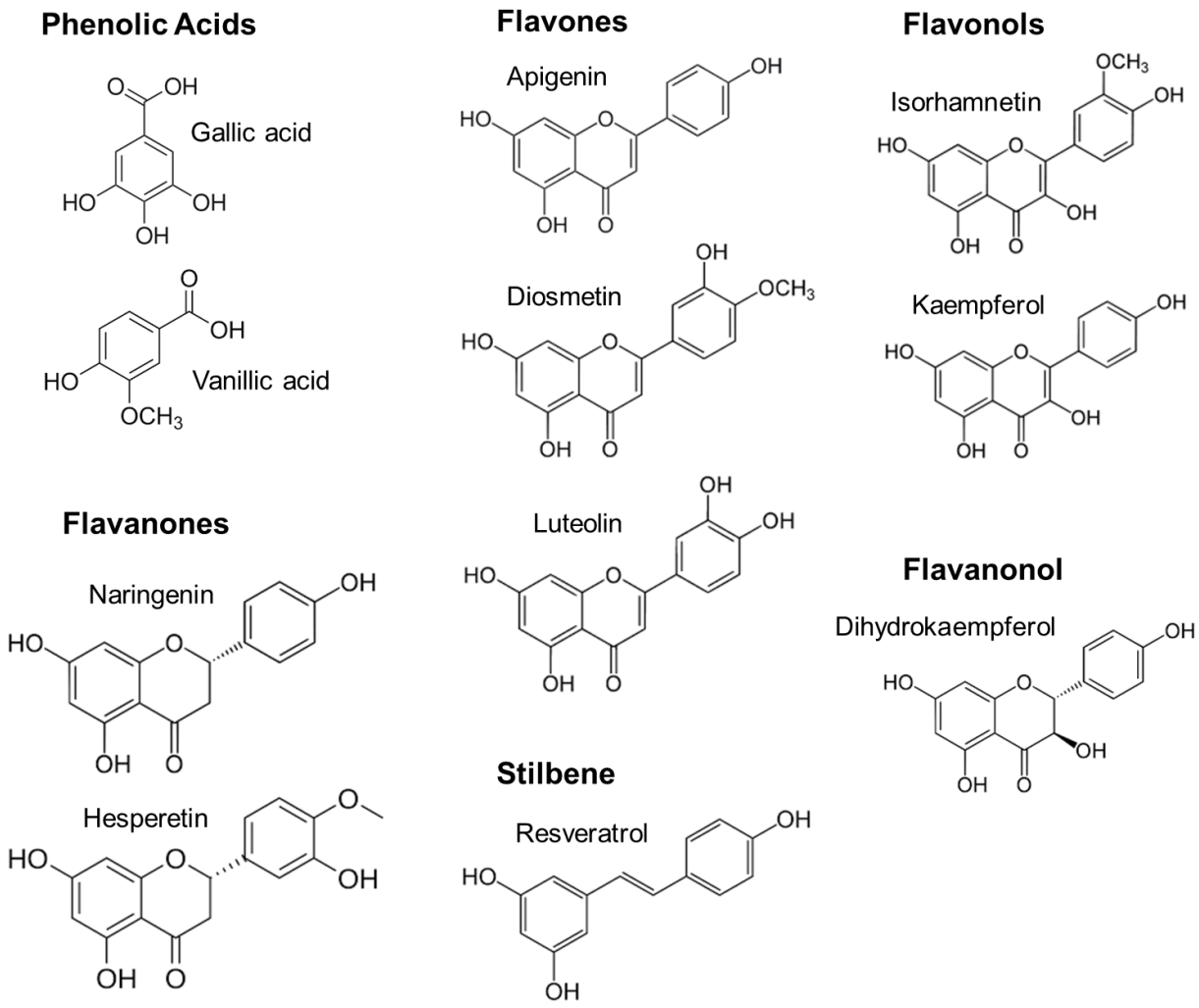


**Fig. 2.2. Chemical structures of important phenolic compounds and examples of each subfamily.**

Lentil (*Lens culinaris* Medikus) is an important grain legume crop that provides a significant source of protein, carbohydrates, and micronutrients for humans. Further, legumes are an important plant functional group since they can form a symbiosis with N-fixing *Rhizobium* bacteria and phosphorus-acquiring arbuscular mycorrhizal fungi (AMF). Some genotypes of annual legume crops have been bred for specific phenotypes, by selection of alterations in the phenylpropanoid pathway, which result in the removal or reduction of tannins from their seed coats. The lentil market class known as zero-tannin (ZT) is determined by expression of the single recessive gene, *tan* (Vaillancourt et al., 1986; Muehlbauer, 2011). Phenolic compound profiling has revealed that myricetin, dihydromyricetin, flavan-3-ols, and proanthocyanidins are only present in the seed coats of normal lentil phenotypes and not in ZT types (Mirali et al., 2016). The

removal or reduction of seed tannins through alteration of a biochemical pathway has the potential to change the tissue chemistry across the entire plant (Tilston et al., 2013), particularly that of lignin, flavanols and phenolic acids present in plant tissues, roots (Bazghaleh et al., 2018) and resulting rhizodeposits (Bekkara et al., 1998; Bazghaleh et al., 2018). However, to my knowledge, no information on decomposition or C dynamics of ZT lentils exists and the extent to which ZT lentils will affect soil microbial processing and CUE remain unclear.

# 3. RHIZOSPHERE MICROBIAL COMMUNITIES AND DISTRIBUTION OF ASSIMILATED CARBON UNDER LENTIL OF DIFFERING BIOCHEMICAL QUALITY

## 3.1. Preface

The decomposition of organic carbon (C) is primarily mediated by soil microorganisms. By partitioning C through anabolic and catabolic processes, soil microorganisms control the flow of C through terrestrial ecosystems. Zero-tannin lentils (ZT) have been selectively bred, through alteration of the phenylpropanoid pathway, to remove tannins from their seed coats. Any alterations in a biochemical pathway of a plant has potential to change the tissue chemistry across the entire plant, including root tissues and rhizodeposits. This chapter quantifies the incorporation of photosynthetically fixed C into the rhizosphere and rhizosphere microorganisms of ZT and traditional tannin containing (TAN) lentil genotypes. With the use of $^{13}C$ stable isotopes, members of the microbial community actively involved in the processing of root-derived C can be identified. This chapter examines the first aspect of C flow through these plant-soil systems—namely, C inputs to the soil—while Chapter 4 looks at the potential C loss from these systems via heterotrophic respiration.

## 3.2. Abstract

Root derived photosynthate is the primary source of readily available C for rhizosphere microorganisms. These microorganisms provide precursors to stabilized soil organic matter (SOM) by transforming plant root exudates into microbial biomass and accelerating the breakdown of aboveground (AG) plant tissues and root detritus. Tannins comprise a large group of plant phenolic secondary metabolites that can alter rhizosphere soil microbial communities. Some annual legume crops have been selectively bred to remove tannins from their seed coats. Any shifts in the biotic processing of rhizodeposits from ZT genotypes have the potential to change pulse crop-derived C trajectories through the soil. We used a $^{13}C$ pulse-labeling procedure to examine the incorporation of root-derived C into individual phospholipid fatty acids (PLFAs) in rhizosphere soils of ZT and TAN lentil genotypes. Microbial biomass was significantly higher under TAN

genotypes (P=0.036). Moreover, unconstrained ordination by NMDS of Bray–Curtis dissimilarity followed by permutational analysis of variance revealed significant differences in community structure between and among ZT and TAN genotypes (tannin, $P<0.0001$; genotype, $P=0.0005$). Specifically, Gram-positive (Gram+) biomarkers (i15:0, a15:0, i16:0, i17:0 and a17:0) dominated ZT genotypes while Gram-negative (Gram-) biomarkers (16:1ω9c, cy17:0, 18:1ω9,7,5c, cy19:0ω9,7c and 20:1ω9c) associated strongly with TAN genotypes. Though both fungal and bacterial biomass were higher in TAN soils, the fungal to bacterial ratio (F:B) remained significantly higher in TAN soils ($P=0.0344$). Activities of the hydrolytic β-glucosidase (BGL) and cellobiohydrolase (CBH) and the oxidative phenol oxidase (PPO) enzymes were assessed. Apart from BGL, significant differences between TAN and ZT genotypes were not found (BGL, $P=0.045$; CBH, $P=0.062$; PPO, $P=0.182$) though generally, extracellular enzyme activities were higher under ZT genotypes. This study has shown that differences in root-derived C under ZT lentil genotypes directly affect microbial community structure and function. Consequently, ZT genotypes of lentil have the potential to alter C partitioning in the soil.

### 3.3. Introduction

Plant roots continuously produce and transfer carbon-rich compounds into the rhizosphere, generating a unique environment with a certain degree of specificity determined by several biotic and abiotic factors. Rhizodeposits consist of exudates, enzyme secretions, lysates from dead cells and mucilage (Grayston et al., 1996). The composition and rate of exudation depend on genetic factors and vary widely among plant species and developmental stage as well as environmental factors (Kochian et al., 2005) such as nutrient availability, soil type, pH, temperature and moisture (Badri and Vivanco, 2009).

Root derived photosynthate is the primary source of readily available C for rhizosphere microorganisms. Microorganisms metabolize organic compounds to satisfy heterotrophic demands for C and energy (Frey et al., 2013). By transforming plant root exudates into microbial biomass and accelerating the breakdown of root detritus, microbial decomposition of organic matter directly links the terrestrial and atmospheric C pools (Houghton, 2007). Decomposition processes partition C by simultaneously returning $CO_2$ to the atmosphere through respiration, while generating other decomposition products that move through varying pathways of SOM formation (Cotrufo et al., 2015). Dissolved organic C (DOC) can sorb directly to mineral soil

particles (Kalbitz et al., 2005), while microbial residues and byproducts of decomposition can become associated with silt and clay sized minerals in the soil (Grandy and Neff, 2008; Cotrufo et al., 2013). Various microorganisms contribute differently to soil C turnover. For example, Gram- bacteria preferentially process C deposited from fresh plant material, whereas Gram+ bacteria are able to utilize more complex C forms (Waldrop and Firestone, 2004; Kramer and Gleixner, 2006). Further, fungi have extensive extracellular enzymatic capabilities and are therefore able to depolymerize macromolecules (Blagodatskaya and Kuzyakov, 2008). Consequently, through its effect on soil microorganisms, the chemical composition of rhizodeposits is important in determining ecological function in the rhizosphere (Cheng and Gershenson, 2007).

Tannins represent a complex range of phenolic compounds that are present in many plant tissues and have diverse physiological and ecological functions (Xie and Dixon, 2005). For example, tannins present in the leaves of many plants provide a means of preventing insect herbivory, while tannins in the seed coats are suggested to function against fungal infection (Caldas and Blair, 2009). Condensed tannins also comprise a large group of plant phenolic secondary metabolites which have been reported to alter rhizosphere soil microbial communities (Tilston et al., 2013). Studies have shown that phenolic acids significantly decrease the functional and genetic diversity of the soil microbial community (Wu et al., 2008) by selectively increasing the species of certain soil microorganisms (Qu and Wang, 2008). Additionally, specific soil microorganisms use phenolic compounds as substrates or signaling molecules (Badri et al., 2013). Conversely, many phenolic compounds have been shown to act as antimicrobials against plant soil-borne pathogens (Sarma and Singh, 2003; Lanoue et al., 2010) and microorganisms with plant growth-promoting abilities (Medina et al., 2011; Wu et al., 2016). Moreover tannins have been reported to inhibit microbial enzyme activities important for nutrient cycling (Appel, 1993), reduce rates of N mineralization thereby inhibiting nitrification (Hättenschwiler and Vitousek, 2000) and altering decomposition of crop residues (Liebeke et al., 2015).

Lentil (*Lens culinaris* Medikus) is an important grain legume crop that provides a significant source of protein, carbohydrates, and micronutrients for humans. Further, legumes are an important plant functional group since they can form a symbiosis with nitrogen (N) fixing *Rhizobium* and phosphorus (P) acquiring arbuscular mycorrhizal fungi (AMF). Some annual legume crops are selectively bred for alterations in the phenylpropanoid pathway, to reduce tannins from their seed coats. The removal or reduction of seed tannins through alteration of a biochemical

pathway has the potential to change the tissue chemistry across the entire plant (Tilston et al., 2013), particularly lignin, flavanols and phenolic acids present in roots (Bazghaleh et al., 2018) and resulting rhizodeposits (Bekkara et al., 1998; Bazghaleh et al., 2018). However, to my knowledge, no information on rhizosphere C dynamics of ZT lentils exists and the extent to which ZT lentils will affect soil microbial processing of C remain unclear.

In this study, the distribution and microbial allocation of root-derived carbon (CdfR, C derived from rhizodeposition) in the rhizosphere of ZT lentil genotypes was examined. A $^{13}CO_2$ pulse labelling technique was used in combination with $^{13}$C-PLFA analysis. The $^{13}CO_2$ pulse labelling technique enables the direct observation of C flow from plants to soil and, linked with PLFA analysis, into the microbial community (Fig. 3.1; Treonis et al., 2004; Denef et al., 2007, 2009). Lentil plants were exposed to $^{13}CO_2$ during plant growth and harvested at flowering to coincide with peak rhizodeposition (Badri and Vivanco, 2009). Carbon stable isotope ratios in PLFAs of soil microorganisms were used to determine the abundance and structure of microbial communities actively assimilating root-derived carbon. The abundance of $^{13}$C in the rhizosphere was determined to estimate the relative contribution of fresh photosynthate to the rhizosphere. It was hypothesized that alterations in the biochemistry of ZT lentils would result in changes of the active microbial population, increase microbial biomass and reduce extracellular enzyme activities in the rhizosphere of ZT lentils.

## 3.4. Material and Methods

### 3.4.1. Seed selection

Three ZT and three TAN lentil genotypes were used in this study. Zero-tannin one (LR-30-32) and TAN 1 (LR-30-101) belong to the lentil recombinant inbred line population LR-30. This population was derived from a cross between the brown seed coat cultivar CDC Robin (genotype Ggc Tgc Tan) and a ZT plant from the breeding line 2670b (genotype Ggc Tgc tan). Both genotypes are homozygous for Tgc and the resulting population have either normal brown (Ggc Tgc Tan) or gray ZT (Ggc Tgc tan) seed coats based on segregation of the dominant or recessive alleles at the Tan locus (Mirali et al. 2016). Zero-tannin one and TAN 1 are therefore genetically related and were chosen to minimize additional genetic effects, allowing differences found to be attributed to the presence or absence of the *tan* gene. Conversely, ZT 2 (ZT4) and TAN

2 (cv. CDC Imvincible) as well as TAN 3 (cv. CDC Maxim) and ZT 3 (cv. CDC Gold) do not share common backgrounds, thus genotypic differences exceed the *tan* locus.

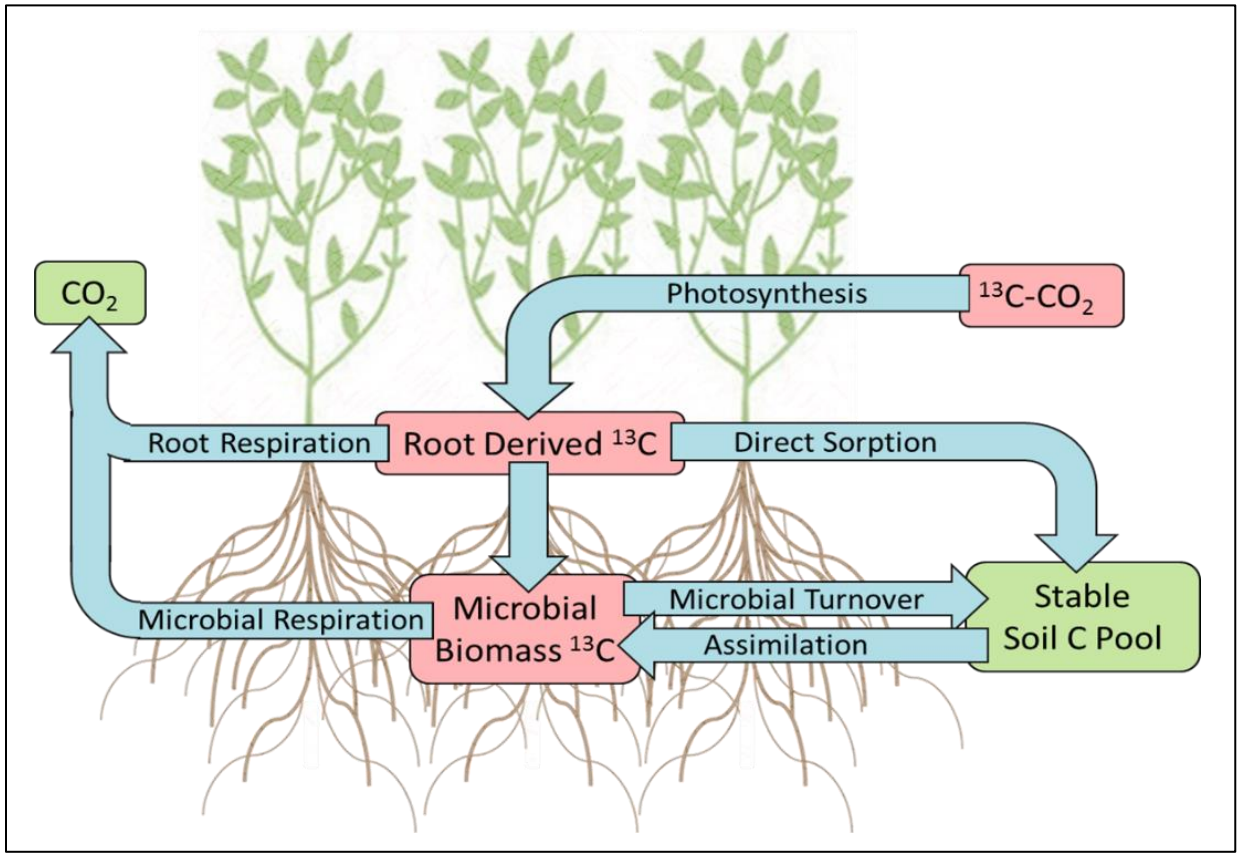


**Fig. 3.1. Schematic representation of carbon (C) flows through terrestrial ecosystems and the application of $^{13}C$ stable isotope probing in tracing C flows through soil microorganisms.**

### 3.4.2. Soil preparation and experimental design

Surface soil (0-15 cm), classified as a Dark Brown Chernozem (Bradwell association), was collected from the Goodale Research Farm in the winter of 2016. The soil was air dried and passed through a 2 mm sieve before mixing with silica sand at a 4:1 soil:sand ratio by weight. The resulting sand-soil mix was classified as a fine sandy loam with a pH of 6.2. The soil-sand mix (415 g) was poured into each plastic (6.9 cm dia. by 20.3 cm deep) 'Deepot cell' (D33L, Stuewe & Sons, Inc.), adjusted to 65% water holding capacity (WHC) with deionized water and allowed two days for even water distribution prior to seeding. Water holding capacity was previously determined by saturating the soils, allowing them to freely drain for 24 h and determining gravimetric water content after oven-drying at 105 °C.

Lentil seeds were germinated at room temperature for three days and planted at a density of one seed per pot. In total, there were four replicate pots for each of the six lentil genotypes subjected to $^{13}C$ labelling and an additional two replicate pots for each genotype grown without $^{13}C$ enrichment to be used as natural abundance controls, therefore n=36. Plants were grown in a growth chamber at 22°C and 18 h of light per day. The experiment was set up as a completely randomized design within each of two sets (one set for $^{13}C$ enrichment and one set for natural abundance controls) and pots were randomized within their respective set every 3 days to ensure homogeneous plant illumination. Pot weight was monitored daily and plants were watered with deionized water every two days to maintain the soil water content at 65% WHC.

### 3.4.3. $^{13}C$ Stable isotope labelling

Atmospheric enrichment of $^{13}C$-$CO_2$ began 15 d after seeding and was carried out bi-weekly thereafter. Pulse-labelling was used to supply $CO_2$ proportional to the photosynthetic rate and achieve uniform and sufficient incorporation of $^{13}C$ into plant structural and metabolic tissues. Each labelling session was 4 h in duration. Labelling took place inside a hermetic acrylic chamber, total $CO_2$ concentration was monitored and maintained using a closed loop system with an infrared gas analyzer (S151 Infrared $CO_2$ Analyzer, Qubit Systems, Kingston, O) (Fig. 3.2). The total $CO_2$ concentration in the chamber was maintained at approximately 400 ppm at a target atmospheric enrichment of 45 Atom% $^{13}CO_2$. Each labelling session consisted of two parts. First, the $CO_2$ in the chamber was allowed to drop to 100 ppm. The ideal gas law was then used to determine the volume (mL) of 99% $^{13}C$-$CO_2$ gas to be injected into the chamber in order to bring $CO_2$ volume (mL) of 99% $^{13}C$-$CO_2$ gas to be injected into the chamber in order to bring $CO_2$ concentration back to 400 ppm. Next, and thereafter, using the resulting atmospheric enrichment, 45% $^{13}C$-$CO_2$ gas was injected into the chamber each time the $CO_2$ concentration dropped below 300 ppm such that an atmospheric enrichment of 45 atom% would be maintained.

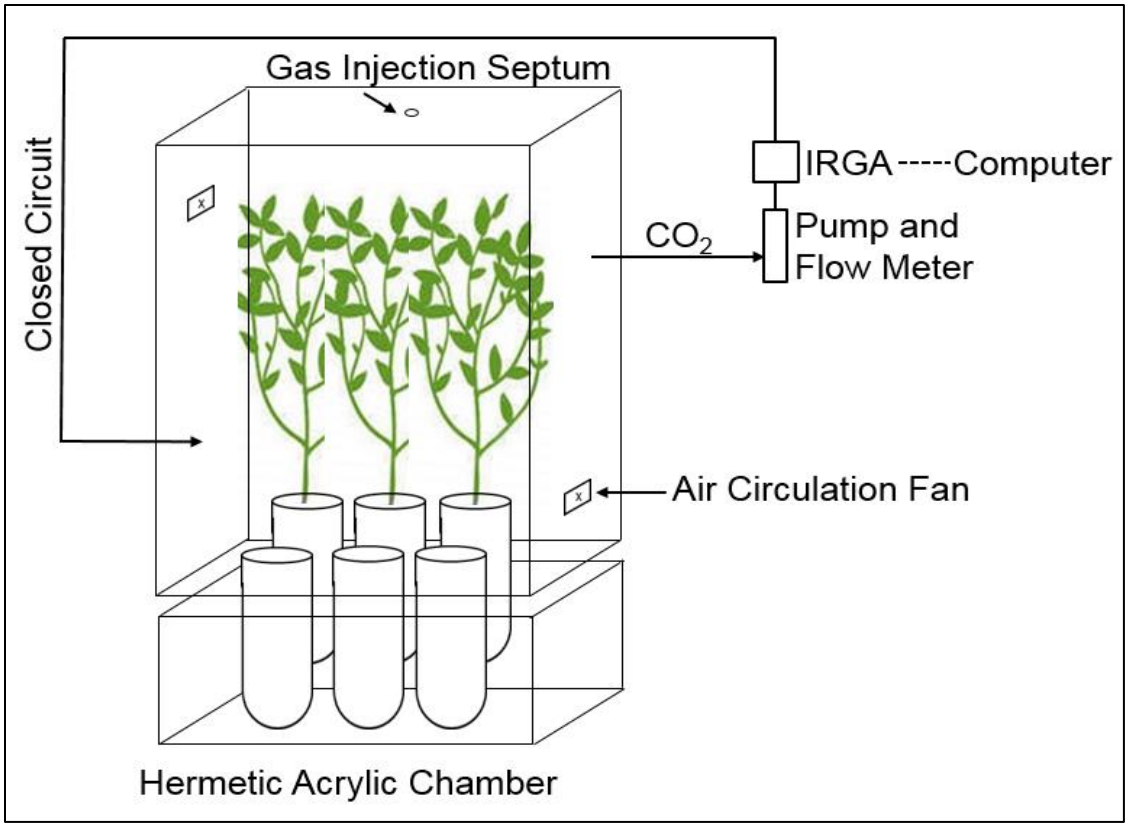


**Fig. 3.2. Schematic representation of a closed loop $^{13}C$ labelling system used to enrich plant-soil-microbe systems with $^{13}C$.**

### 3.4.4. Plant harvest and sample analysis

Plants were harvested 42 d after seeding. Roots had proliferated throughout the entire pot and as such, all soil was considered to have interacted with roots. Aboveground plant tissues were cut at the surface of the soil. Roots were then removed from the soil; a sub-sample of soil was kept frozen at -20°C and used for $^{13}C$-PLFA analysis and for the determination of extracellular enzyme activities. Roots were washed with 100 mL of 0.05M NaCl for 15 min and any remaining rhizosphere soil was recovered from the washed roots. Following shaking, roots were removed from the flask and rinsed multiple times with tap water, while the remaining soil-buffer slurry was centrifuged at 5000 rpm for 15 minutes. The soil pellet was freeze dried, while aboveground plant tissues, washed roots and soil were air dried at room temperature. All dried plant and soil compartments were finely ground in a ball mill and assessed for atom% $^{13}C$ using a DeltaPLUS isotope ratio mass spectrometer (Thermo Fisher Scientific, Waltham, MA) coupled with a Hewlett Packard 6890 Series II GC (Agilent, Palo Alto, CA) through a GC Combustion-III interface

(Thermo Fisher Scientific, Waltham, MA). The proportion of rhizosphere C derived from roots (CdfR) was calculated according to Sangster (2010) and is as follows:

$$\%CdfR = (\text{atom\% } {}^{13}C_{\text{Enriched soil}} - \text{atom\% } {}^{13}C_{\text{Natural abundance soil}})/(\text{atom\% } {}^{13}C_{\text{Enriched root}} - \text{atom\% } {}^{13}C_{\text{Natural abundance root}}) \quad \text{(Eq. 3.1)}$$

*3.4.4.1. Extracellular enzyme activities*

Activities of extracellular enzymes involved in the hydrolysis and oxidation of organic compounds important to soil C cycling were determined on thawed soils previously stored at -20 °C. The hydrolytic enzymes β-glucosidase (BGL) and cellobiohydrolase (CBH) were assayed based on the fluorometric determination of 4-methylumbelliferone (MUB) released from synthetic substrates (Bell et al., 2013). The oxidative phenol oxidase (PPO) was assayed based on colorimetric determination of L-3,4-dihydroxy phenylalanine (L-DOPA). The substrates used were 25 mM MUB-β-D-glucopyranoside, 25 mM MUB-β-D-cellobioside and 10 mM L-DOPA for determination of BGL, CBH and PPO respectively. Soil aliquots (1.0 g) were combined with 125 mL 50 mM sodium acetate buffer, pH 6.2, and homogenized in a blender for 1 min. The resulting suspensions were continuously stirred on a magnetic stir plate while 1.8 mL aliquots were dispensed into 5 mL centrifuge tubes. Assayed samples received 0.45 mL of substrate. Blank tubes received 0.45 mL of acetate buffer plus 1.8 mL of sample suspension. Negative controls received 0.45 mL substrate solution plus 1.8 mL of acetate buffer. Quench standards received 0.45 mL of standard plus 1.8 mL sample suspension. Reference standards received 0.45 mL of standard plus 1.8 mL acetate buffer. Quench standards were not used for PPO. Tubes were incubated at 21 °C in the dark while shaking at 140 rpm for 2.5 h (BGL and CBH) or still for 20 h (PPO). To stop the reaction, tubes were centrifuged at 4 °C. Fluorescence (excitation 360 nm, emission 465 nm) or absorbance (460 nm) in the supernatant was measured on a microplate reader (F5 Multi-Mode Microplate Reader, Molecular Devices, San Francisco, CA). Hydrolytic enzyme activities were determined using a standard curve of MUB with concentrations ranging between 0 and 100 μM. All enzyme activities were expressed in units of nmol substrate consumed $g^{-1}$ dry weight $h^{-1}$.

*3.4.4.2. Microbial PLFA extraction and analysis*

Phospholipid fatty acids (FAs) were used as a measure of viable microbial biomass and determination of microbial community composition and were extracted as outlined by Helgason et al. (2010). Briefly, lipids were extracted from 4 g ground freeze-dried soil using a phosphate buffered solution. Phospholipids, neutral lipids and glycolipids were collected in the organic phase and fractioned using a solid phase extraction column (0.50 g Si; Varian Inc., Mississauga, ON). Phospholipids were methylated by alkaline methanolysis to produce fatty acid methyl esters (FAMEs) and analyzed by GC- FID (Scion 436-GC, Scion Instruments, Livingston, WL). Peaks were identified by comparing Kovats indices with retention times normalized to fatty acid standards and quantified based on the addition of internal standard methyl nonadecanoate (19:0). Fatty acids were separated into microbial groups according to Arcand et al. (2016). Total biomass was calculated as the sum of all identified peaks. Bacterial biomass was assessed using 18 biomarkers (i14:0, i15:0, a15:0, 15:1ω6c, i16:0, 16:1ω9c, 16:1ω7c, i17:0, a17:0, cy17:0, i18:0, 18:1ω7c, 18:1ω9c, 18:1ω5c, cy19:0, 20:1ω9c, 10Me16:0, 10Me18:0). Bacteria were separated into Gram+ (i14:0, i15:0, a15:0, i16:0, i17:0, a17:0, i18:0) and Gram- (15:1ω6c, 16:1ω9c, 16:1ω7c, cy17:0, 18:1ω9c, 18:1 ω7c, 18:1 ω5c, cy19:0, 20:1ω9c). Saprotrophic and AM fungi were identified using the 18:2ω6c and 16:1ω5c biomarkers respectively. Finally, biomarkers 10Me16:0 and 10Me18:0 were used as indicators of actinobacteria.

Carbon isotope ratios of individual PLFAs were determined using a DeltaPLUS isotope ratio mass spectrometer (Thermo Fisher Scientific, Waltham, MA) coupled with a Hewlett Packard 6890 Series II GC (Agilent, Palo Alto, CA) through a GC Combustion-III interface (Thermo Fisher Scientific, Waltham, MA). A reference $CO_2$ sample of known isotopic composition was injected at the beginning and end of each sample run and a standard mixture of eight FAs of known isotopic composition was run with every analysis. Total PLFA analysis by GC-FID identified 30 FAMEs and isotope ratios were obtained for 19 of them. The quantity of $^{13}C$ incorporated into individual PLFA biomarkers was calculated as:

$$^{13}C_{inc} = (F_{enriched} - F_{natural\ abundance}) * PLFA \qquad \text{(Eq. 3.2)}$$

where PLFA is the amount (μg $g^{-1}$ soil) of an individual lipid as determined by GC-FID and F is the fractional abundance of $^{13}C$ calculated as:

$$F = {}^{13}C/({}^{13}C + {}^{12}C) = R/(R + 1) \quad \text{(Eq. 3.3)}$$

The carbon isotope ratio (R) was derived by GC-C-IRMS measurement, relative to the international Vienna PeeDee Belemnite (VPDB) standard, 0.0111802 ± 0.0000090, as follows:

$$R = (\delta^{13}C/1000 + 1) * R_{VPDB} \quad \text{(Eq. 3.4)}$$

Delta values ($\delta^{13}C$) were adjusted to account for dilution with $^{13}C$ depleted MeOH during derivatization as follows:

$$\delta^{13}C_{PLFA} = [(C_n + 1) * \delta^{13}C_{FAME} - \delta^{13}C_{MeOH}]/ C_n \quad \text{(Eq. 3.5)}$$

where $C_n$ is the number of carbon atoms in the PLFA and $\delta^{13}C_{MeOH}$ is the delta value for MeOH (-43.96). The percent distribution of incorporated $^{13}C$ into individual biomarkers or functional groups, as a proportion of the total $^{13}C$ incorporated into all fatty acids in the community was calculated as follows:

$$\%\ {}^{13}C_{dist} = ({}^{13}C - PLFA/ \sum {}^{13}C - PLFA) * 100 \quad \text{(Eq. 3.6)}$$

### 3.4.5. Statistical analysis

The effects of genotype and tannin content on PLFA abundance and enzyme activity were assessed with a linear mixed model using the lme function in the nlme package in R. Tannin type and genotype were fixed factors and genotype was nested within tannin. Means were compared using Tukey's HSD post-hoc test. Community structure analysis was performed using NMDS of Bray-Curtis dissimilarities on log(mol%+1) transformed PLFA data. Significance testing was performed with permutational analysis of variance using the ADONIS function in the Vegan 2.5-

3 package (Oksanen et al., 2018) in R. Apart from community structure data, all data met ANOVA assumptions of normality and homogeneity of variance according to Shapiro Wilk and Levene's test, respectively. All statistical analyses were performed using R .3.5.1 (R Development Core Team, 2018).

## 3.5. Results

### 3.5.1. Microbial community structure and abundance

Total PLFA abundance, bacteria, and fungi were significantly higher in TAN soils than ZT soils ($P$=0.036, $P$=0.002, $P$=0.001; Fig. 3.3). Though both fungal and bacterial biomass were higher in TAN soils, the F:B was also significantly higher in TAN soils ($P$=0.042). Community structure of total ($^{12}C$ + $^{13}C$) PLFAs showed a distinct pattern associated with TAN and ZT genotypes (Fig. 3.4 A); however, this pattern became less distinct among actively $^{13}C$-assimilating microorganisms (Fig. 3.4 B). Nevertheless, Gram- bacteria and fungi were more prominent in TAN soils while Gram+ bacteria and AMF were primarily associated with ZT soils (Table 3.1).

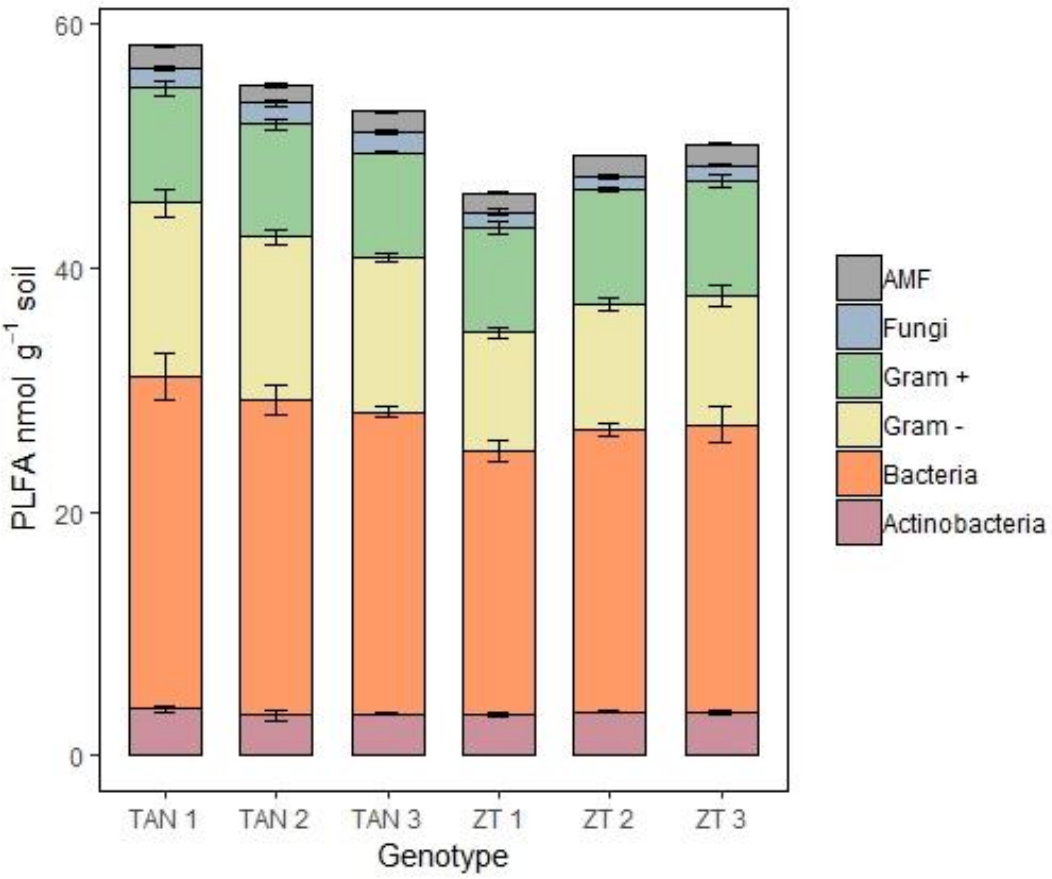


**Fig. 3.3. Total PLFA biomass and abundance within individual microbial groups in soils under tannin (TAN) and zero-tannin (ZT) lentil genotypes (n=6 and is a combination of enriched, n=4 and natural abundance controls, n=2).**

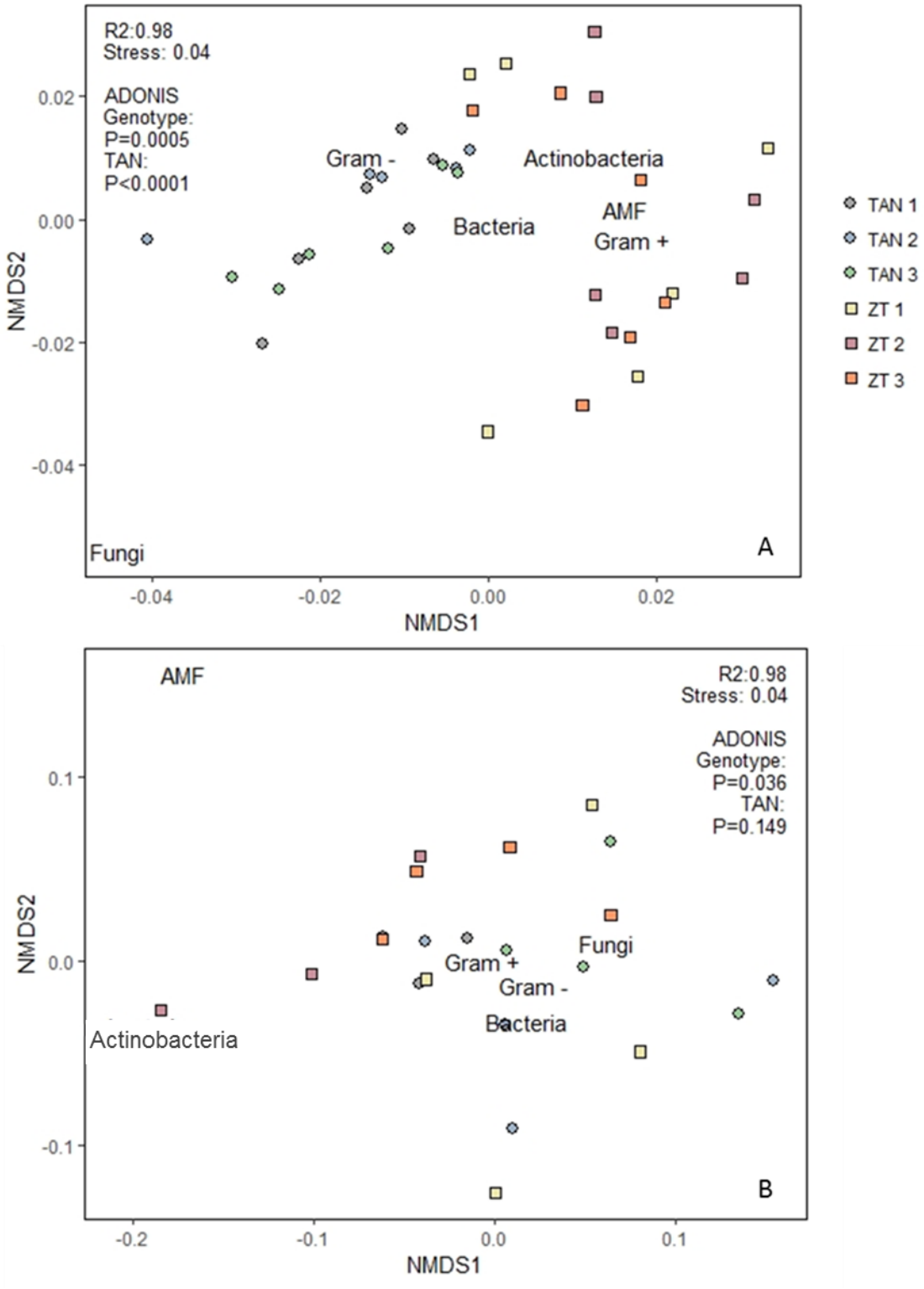


**Fig. 3.4. Non-metric multidimensional scaling of Bray–Curtis dissimilarities indicating total PLFA ($^{12}C$ + $^{13}C$) (A) and $^{13}C$ enriched (B) PLFA profiles in soils under tannin (TAN) and zero-tannin (ZT) lentil genotypes. Total PLFA, n=6 and $^{13}C$ enriched PLFA, n=4.**

### 3.5.2. $^{13}$C incorporation and distribution in PLFAs, soil and roots

The $^{13}$C label incorporation into microbial PLFA groups, is presented in Table 3.1. Label incorporation into total PLFA biomass between TAN and ZT soils was statistically significant ($P$=0.002), with TAN soils consistently showing higher label incorporation than ZT soils. Of the $^{13}$C incorporated into PLFAs, approximately 70% was distributed among bacteria and 25% in fungi (Table 3.2). Because the fungal group is represented by only one PLFA biomarker, this likely indicates a fungal dominance in the rhizosphere. Tannin had a significant effect on distribution in Gram+ bacteria and AMF (Gram+, $P$=0.035; AMF, $P$= 0.023). In both TAN and ZT soils, Gram- bacteria incorporated over 4.5 times more label than Gram+ bacteria. Enrichment of aboveground plant material, roots, loosely- and closely-adhering (i.e., recovered from soil-buffer slurry during root washing) rhizosphere soil was 7.58, 4.99, 1.15 and 1.21 atom% $^{13}$C respectively (Table 3.3). Differences between genotypes were not significant. Percent CdfR was calculated as the proportion of $^{13}$C in soil relative to $^{13}$C in the roots; it comprised 1.91% and 3.26% in the loosely- and closely-adhering rhizosphere soils, respectively. However, CdfR in any of the soil fractions was not significantly different between genotype. Likewise, aboveground and belowground plant biomass were not found to be significantly different among genotypes nor between TAN and ZT genotypes (Table 3.3).

**Table 3.1. $^{13}C$ label incorporation into individual PLFA microbial groups in soils under zero-tannin (ZT) and tannin (TAN) genotypes of lentil.**

| Genotype | Total Biomass | Bacteria | Fungi | Gram+ | Gram- | AMF | Actinobacteria |
|---|---|---|---|---|---|---|---|
| | | | | μg $^{13}C$ $g^{-1}$ soil | | | |
| TAN 1 | 0.82 ± 0.05† | 0.57 ± 0.05 | 0.22 ± 0.02 | 0.12 ± 0.01 | 0.43 ± 0.04 | 0.03 ± 0 | 0.01 ± 0 |
| TAN 2 | 1.23 ± 0.21 | 0.87 ± 0.15 | 0.34 ± 0.10 | 0.13 ± 0.01 | 0.72 ± 0.14 | 0.02 ± 0.01 | 0.01 ± 0 |
| TAN 3 | 1.27 ± 0.16 | 0.84 ± 0.09 | 0.40 ± 0.08 | 0.12 ± 0.02 | 0.71 ± 0.07 | 0.03 ± 0.01 | 0.01 ± 0 |
| ZT 1 | 0.73 ± 0.16 | 0.48 ± 0.07 | 0.23 ± 0.09 | 0.08 ± 0.01 | 0.39 ± 0.06 | 0.02 ± 0.01 | 0.01 ± 0 |
| ZT 2 | 0.68 ± 0.06 | 0.45 ± 0.04 | 0.19 ± 0.07 | 0.09 ± 0.01 | 0.35 ± 0.02 | 0.04 ± 0.02 | 0.01 ± 0 |
| ZT 3 | 0.70 ± 0.12 | 0.45 ± 0.07 | 0.22 ± 0.05 | 0.09 ± 0.01 | 0.35 ± 0.07 | 0.03 ± 0.01 | 0.01 ± 0 |
| *P* Value‡ | | | | | | | |
| Tannin | 0.002 | <0.001 | 0.078 | 0.003 | <0.001 | 0.665 | 0.618 |
| Genotype | 0.206 | 0.148 | 0.481 | 0.899 | 0.092 | 0.331 | 0.218 |

† Mean values followed by ± standard error, n=4
‡ Based on Tukey's honestly significant difference test

**Table 3.2. Percent $^{13}C$ distribution among individual PLFA microbial groups in soils under tannin (TAN) and zero-tannin (ZT) lentil genotypes.**

| Genotype | Bacteria | Fungi | Gram+ | Gram- | AMF | Actinobacteria |
|---|---|---|---|---|---|---|
| TAN 1 | 69.51 ± 1.72a† | 26.83 ± 1.63a | 14.63 ± 0.8a | 52.44 ± 1.75a | 3.66 ± 0.19ab | 1.22 ± 0.43a |
| TAN 2 | 70.73 ± 3.86a | 27.64 ± 3.98a | 10.57 ± 1.18a | 58.54 ± 4.12a | 1.63 ± 0.75a | 0.81 ± 0.40a |
| TAN 3 | 66.14 ± 2.88a | 31.50 ± 3.13a | 9.45 ± 0.61a | 55.91 ± 2.69a | 2.36 ± 0.86a | 0.79 ± 0.10a |
| ZT 1 | 65.75 ± 5.90a | 31.51 ± 5.58a | 11.00 ± 1.25a | 53.42 ± 4.51a | 2.75 ± 1.05a | 1.37 ± 0.19a |
| ZT 2 | 66.17 ± 4.12a | 27.94 ± 5.13a | 13.24 ± 0.51a | 51.47 ± 3.61a | 5.88 ± 1.46b | 1.47 ± 0.50a |
| ZT 3 | 64.29 ± 1.44a | 31.43 ± 1.63a | 12.90 ± 1.72a | 50.00 ± 1.24a | 4.29 ± 0.72ab | 1.43 ± 0.46a |
| | | | | | | |
| *P* Value | | | | | | |
| Tannin | 0.624 | 0.983 | 0.035 | 0.335 | 0.023 | 0.231 |
| Genotype | 0.941 | 0.979 | 0.150 | 0.695 | 0.006 | 0.084 |

† Mean values, n=4, (±SE) followed by similar letters within each microbial group are not significantly different at $P > 0.05$ based on Tukey's HSD.

**Table 3.3. Atom % $^{13}C$ of enriched plant and soil components, carbon derived from roots (CdfR) and plant biomass from zero-tannin (ZT) and tannin (TAN) genotypes of lentil.**

| Genotype | Atom % $^{13}C$ | | | | % CdfR† | | Biomass‡ (g) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Shoot | Root | LAR§ | CAR¶ | LAR | CAR | Shoot | Root | S:R# |
| TAN 1 | 7.35 ± 0.16†† | 4.68 ± 0.25 | 1.15 ± 0.01 | 1.20 ± 0.02 | 1.74 ± 0.31 | 3.39 ± 0.51 | 0.42 ± 0.06 | 0.52 ± 0.11 | 0.81 |
| TAN 2 | 7.66 ± 0.14 | 5.21 ± 0.06 | 1.18 ± 0.01 | 1.21 ± 0.01 | 2.36 ± 0.42 | 3.31 ± 0.45 | 0.39 ± 0.08 | 0.55 ± 0.15 | 0.71 |
| TAN 3 | 7.40 ± 0.17 | 5.07 ± 0.13 | 1.17 ± 0.01 | 1.22 ± 0.01 | 2.17 ± 0.31 | 3.49 ± 0.33 | 0.36 ± 0.05 | 0.56 ± 0.10 | 0.64 |
| ZT 1 | 7.74 ± 0.24 | 5.35 ± 0.09 | 1.13 ± 0.01 | 1.21 ± 0.03 | 1.26 ± 0.18 | 3.22 ± 0.85 | 0.32 ± 0.07 | 0.46 ± 0.13 | 0.70 |
| ZT 2 | 7.57 ± 0.22 | 5.05 ± 0.08 | 1.17 ± 0.02 | 1.21 ± 0.02 | 2.43 ± 0.36 | 3.18 ± 0.33 | 0.40 ± 0.07 | 0.60 ± 0.07 | 0.67 |
| ZT 3 | 7.75 ± 0.15 | 4.63 ± 0.11 | 1.13 ± 0.02 | 1.18 ± 0.01 | 1.51 ± 0.49 | 2.94 ± 0.32 | 0.41 ± 0.02 | 0.44 ± 0.09 | 0.93 |
| *P* Value | | | | | | | | | |
| Tannin | 0.90 | 0.99 | 0.47 | 0.78 | 0.12 | 0.64 | 0.84 | 0.59 | |
| Genotype | 0.10 | 0.97 | 0.71 | 0.97 | 0.25 | 0.97 | 0.79 | 0.88 | |

† Calculated as the difference between control and enriched samples divided by the difference between control and enriched roots, harvested at flowering, n=4
‡ n=6 and includes $^{13}C$ enriched and natural abundance controls
§ Loosely-adhering rhizosphere soil
¶ Closely-adhering rhizosphere soil
# Shoot to root ratio
†† Mean values followed by ± standard error

### 3.5.3. Extracellular enzyme activity

Averaged across genotypes, extracellular enzyme activities were higher in soils under ZT lentil genotypes than in soils under TAN genotypes (Fig. 3.5). Genotype and tannin content significantly affected BGL activity (genotype, *P*=0.002; tannin, *P*=0.0102), with BGL being 30% higher under the ZT 2 genotype compared to all others. Apart from BGL, genotype and tannin content had no significant effect on extracellular enzyme activity; though CBH activity tended to be higher under ZT soils (*P*=0.087*).*

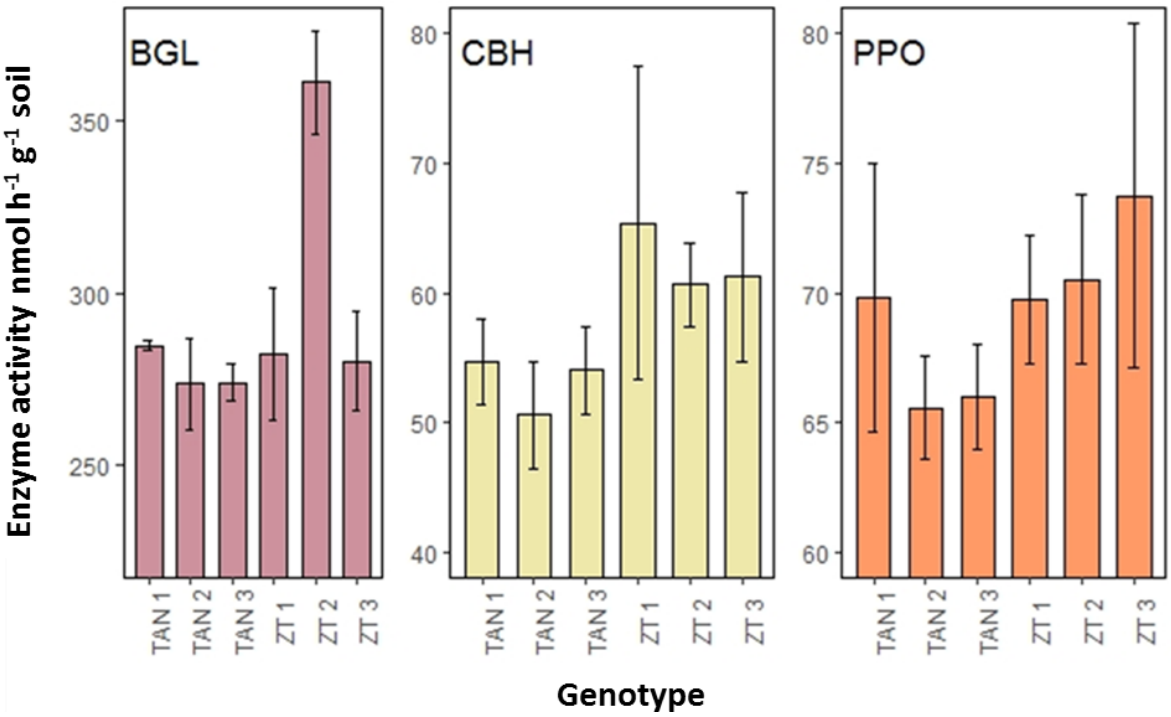


**Fig. 3.5. Activities (nmol h $^{-1}$ g $^{-1}$) of cellobiohydrolase (CBH), β-glucosidase (BGL) and polyphenol oxidase (PPO) in soils under tannin (TAN) and zero-tannin (ZT) lentil genotypes. Bars represent standard error of biological replicates (n=4).**

### 3.6. Discussion

With the use of $^{13}$C isotope labelling and PLFA analysis, the fate and distribution of root derived $^{13}$C was quantified among $^{13}$C-PLFA biomarkers and rhizosphere soils between ZT and TAN genotypes of lentil. Both the total PLFA abundance and the active microbial groups utilizing root derived C differed between TAN and ZT lentil genotypes. Though no studies have directly

assessed the effects of ZT lentils on rhizosphere microbial communities, some have shown ZT lentils to have altered polyphenol expression in root tissues (Bazghaleh et al., 2018) and ZT faba beans to have different exudation patterns (Bekkara et al., 1998; Bazghaleh et al., 2018) when compared to traditional TAN genotypes. Therefore, differences in microbial community structure are likely due to changes in rhizodeposition.

Gram-positive bacteria are important in the decomposition of SOM (Kramer and Gleixner, 2006, 2008) and are capable of utilizing complex C forms (Fanin et al., 2019), while Gram- bacteria (Treonis et al., 2004; Creamer et al., 2016) and fungi (Denef et al., 2009; Tavi et al., 2013) are associated with the utilization of simple C compounds and fresh input. Total $^{13}C$ label incorporation into PLFAs was significantly greater in TAN soils, indicating that microorganisms in TAN soils are actively assimilating more root derived C. Thus, the dominance of Gram+ bacteria in ZT soils and Gram- bacteria and fungi in TAN soils likely suggests a greater availability of simple C forms in the rhizosphere of TAN soils. In accordance with other studies (Denef et al., 2009; Tavi et al., 2013), $^{13}C$ label incorporation into Gram+ bacteria and actinobacteria was significantly lower than Gram- bacteria and fungi in both TAN and ZT soils, further indicating that Gram+ bacteria do not preferentially utilize fresh C input. Though %CdfR was not significantly different between TAN and ZT genotypes, total microbial abundance was higher in TAN soils suggesting that microorganisms in ZT soils likely follow K growth strategies, while microorganisms in TAN soils follow R growth strategies (Grayston et al., 1996; Fierer et al., 2007; Berg and Smalla, 2009; Dorodnikov et al., 2009). Moreover, as neither %CdfR nor root biomass were significantly different, it is likely the quality, not quantity, of rhizodeposition that differs between TAN and ZT soils. Examination of root tissues and root extracts by liquid chromatography-mass spectrometry (LC-MS) (Bazghaleh et al., 2018) revealed elevated levels of phenolic compounds in the lentil genotype ZT4 compared to the TAN genotypes Eston and CDC Maxim. Specifically, increased amounts of diosmetin and gallic acid were found in the root extracts of ZT4 and apigenin, apigenin 7-glucoside, hesperitin 7-rutinoside, narigenin, narigenin 7-rutinoside, dihydrokaempferol and vanillic acid 4-glucoside in the root tissues of ZT4. These compounds contain three aromatic rings in their base structure while resveratrol (greater amounts detected in tannin genotypes Eston and CDC Maxim), contains only two aromatic rings and may be considered a relatively simpler compound. Additional N may promote the synthesis of extracellular enzymes that break down more complex compounds (Allison et al., 2009) thus, the

higher enzyme activities in ZT soils might be partially attributed to increased N availability or the greater concentration of complex compounds in present in the rhizosphere and, combined with lower PLFA biomass, may cause microorganisms to mine SOM for nutrients.

Root secreted polyphenols play critical roles in shaping rhizosphere microbial communities by acting as specific substrates, signaling molecules or antimicrobials (Badri et al., 2013). As flavonoids are present in root exudates, their involvement in the signaling of the host plant-AMF interactions has been suggested by many and summarized (Morandi, 1996; Vierheilig et al., 1998). Conversely, Becard (1994) demonstrated that root metabolites in addition to flavonoids may stimulate AMF growth and that flavonoids are not necessary for the establishment of mycorrhizal symbiosis. Therefore, it is not surprising that AMF was found to associate to a higher degree in soils of ZT lentil genotypes, where flavanoid synthesis has been altered. Rhizobia are Gram- bacteria associated symbiotically with the roots of leguminous plants (Peña et al., 2017) and it is well documented that Rhizobia species have the ability to utilize phenolic acids as a carbon source (Van Rossum et al., 1995; Irisarri et al., 1996). Moreover, the process of nodule formation in legumes involves the production of flavonoids in the seed and root exudates of the plant to serve as chemoattractants that guide rhizobial cells to legume root hairs (Steinkellner et al., 2007) and as nodulation signals to the microbial symbiont (Phillips and Torrey, 1972). The higher relative proportion of Gram- bacteria in TAN soils compared to ZT soils may be indicative of the lack of flavanoid signaling in ZT soils, and may lead to reduced nodule formation during plant growth.

### 3.7. Conclusion

In this study, with the use of $^{13}C$ stable isotope labelling, the fate and distribution of root derived C were examined in rhizosphere soils and microbial communities of ZT and TAN genotypes of lentil. Though %CdfR was not significantly different between ZT and TAN genotypes, total PLFA biomass and community composition differed significantly, thus indicating a difference in the quality of root derived C between ZT and TAN genotypes. While ZT soils were primarily dominated by oligotrophic organisms, TAN soils were dominated mainly by copiotrophic microorganisms, likely indicating the relative simplicity of root derived C in TAN genotypes compared to ZT genotypes. Limitations in substrate availability in ZT soils may cause microorganisms to mine SOM for nutrients via inefficient mechanisms (Geyer et al., 2016) thus altering soil carbon cycling and C trajectories through the soil.

# 4. MICROBIAL DECOMPOSITION AND CARBON DYNAMICS IN SOILS AMENDED WITH PLANT AND ROOT TISSUES OF VARYING BIOCHEMICAL QUALITIES

## 4.1. Preface

Soil organic matter (SOM) is the largest carbon (C) stock in terrestrial ecosystems. This C has the potential to act as either a sink or source for $CO_2$, depending on the balance between accumulation and decomposition of organic material. The decomposition of organic C is primarily mediated by soil microorganisms. By partitioning C through anabolic and catabolic processes, soil microorganisms control the flow of C through terrestrial ecosystems. Some annual legume crops have been selectively bred, for alterations in the phenylpropanoid pathway, to reduce tannins from their seed coats. Any alterations in a biochemical pathway of a plant has potential to change the tissue chemistry across the entire plant. In Chapter 3, $^{13}C$ stable isotopes were used to examine the incorporation of root derived C into phospholipid fatty acids (PLFAs) in rhizosphere soils of zero-tannin (ZT) and traditional tannin containing (TAN) lentil genotypes of. Significant differences in microbial community composition and extracellular enzyme activity between ZT and TAN genotypes were found, indicating a shift in the biotic processing of rhizodeposits from ZT genotypes. In this chapter, experimentation was designed to determine if differences in biotic processing were limited to rhizodeposits or if differences in tissue chemistry between ZT and TAN genotypes would lead to changes in the microbial decomposition of aboveground (AG) and belowground (BG) plant detritus, as this has the potential to change pulse crop-derived C trajectories through the soil.

## 4.2. Abstract

Soil microbial carbon use efficiency (CUE) is the relative partitioning between microbial anabolic and catabolic processes; this partitioning plays a critical role in C storage, nutrient availability, and SOM formation—thus affecting C trajectories in the soil. Soil microcosms were incubated with AG and BG residues originating from ZT and TAN genotypes of lentil to assess the effect of residue biochemical quality on decomposition C dynamics. Soils with AG residues

respired both faster and cumulatively more $CO_2$-C than soils with BG residues. Differences between ZT and TAN genotypes were only observed at the onset of decomposition. Total microbial biomass, as determined by chloroform fumigation-extraction, was higher in AG than BG soils and differences between genotypes were not observed. Microbial metabolic quotients were determined using cumulative $CO_2$-C respired and total MBC. Soil microbial communities in microcosms incubated with BG residues produced proportionately less $CO_2$ per g residue degraded than microcosms incubated with AG soils. Furthermore, unconstrained ordination by NMDS of Bray–Curtis dissimilarity followed by PERMANOVA revealed significant differences in microbial community structure between AG and BG soils. Gram-positive (Gram+) bacteria, notably actinobacteria, were relatively more prominent in control and BG soils, while Gram-negative (Gram-) bacteria, arbuscular mycorrhizal fungi (AMF) and saprotrophic fungi were relatively more dominant in AG soils. Though not significantly different, extracellular enzyme activities were generally highest in control soils, followed by BG soils. Further, enzyme activities peaked on day 29 of the incubation and decreased with time. No differences in enzyme activities were observed between genotypes. I have shown here that biochemically complex compounds have the potential to be degraded with a better efficiency than what has been previously thought and that this difference is mediated by the soil microbial communities. Moreover, biochemical alterations related to plant genotype were too slight to substantially affect microbial processing of plant residues, suggesting that C:N are more important in regulating decomposition dynamics than these differences in chemical structure.

### 4.3. Introduction

Soil organic matter is the largest C stock in terrestrial ecosystems, containing three times the amount of C than the atmosphere and four times the amount stored in all living plants and animals (Lal et al., 2015; Berhongaray et al., 2018). Organic matter has the potential to act as either a sink or source for $CO_2$, depending on the balance between accumulation and decomposition of organic material. Historically, differences in the decomposability of plant tissues were primarily attributed to differences in their biochemical composition (Lehmann and Kleber, 2015). For example, roots contain higher amounts of lignin, suberin, polyphenols and tannins than AG plant residues (Melillo et al., 1982; Bertrand et al., 2006; Castellano et al., 2015) and would be expected to decompose at lower rates (Bloomfield et al., 1993; Rasse et al., 2005; Fujii and Takeda, 2010;

Kleber, 2010) thus contributing more to the stable soil C pool (Rasse et al., 2005; Johnson et al., 2014). Indeed, empirical evidence is building against the notion of intrinsic biochemical recalcitrance (Jenkinson et al., 1985; Kögel-Knabner et al., 2008; Marschner et al., 2008; Kleber and Johnson, 2010) as the governing factor of SOM formation and it is now believed that it is the inaccessibility to enzymes, not recalcitrance (Dungait et al., 2012; Lehmann and Kleber, 2015) that is responsible for the slow degradation of C compounds in soil. Further, recent studies have indicated that labile residues will decompose at a faster rate than biochemically complex residues initially, but store more C in the soil in the form of microbial byproducts and necromass (Cotrufo et al., 2013; Kallenbach et al., 2016).

Both substrate quality and microbial community composition are important factors governing microbial CUE (Manzoni et al., 2012; Sinsabaugh et al., 2013). Microbial CUE is the relative partitioning of substrate-C between catabolic respiration and anabolic processes that support growth, thus controlling the flow of C through terrestrial ecosystems. Microbial community composition can affect CUE as a result of varied life strategies (Fierer et al., 2007). For example, copiotrophic microorganisms thrive in nutrient rich environments and are characterized by high growth rates and fast turnover. By contrast, oligotrophs are characterized by their ability to grow under low substrate concentrations, and generally possess a higher substrate utilization efficiency (Fierer et al., 2007; Roller and Schmidt, 2015). Therefore, structurally different substrates can be processed by the same community with varying efficiencies.

Both AG and BG plant tissues are recognized as major contributors to SOM (Cotrufo et al., 2013), though there are contrasting accounts surrounding the primary source of C to the mineral soil. Some studies have shown the primacy of roots in forming the stable soil C pool (Austin et al., 2017; Sokol and Bradford, 2019). However, root detritus and exudates could not be partitioned in these field studies; thus not explicitly explaining the role of root detritus in the formation of SOM. Sokol et al. (2018) describe that it is the location and accessibility of C to microorganisms that determines the fate of C in the soil, though still not directly comparing AG and BG tissues in decomposition. Further, studies have shown that BG residues are respired at lower rates than AG residues (Silver and Miya, 2001; Wang et al., 2010; Birouste et al., 2012), yet this information is based solely on mass loss kinetics and we still lack knowledge based on mechanisms controlling the decomposition of AG and BG detritus. Other studies have shown that AG inputs significantly contribute to the stable soil C pool (Michalzik et al., 2003; Kalbitz and Kaiser, 2008), thus further

confounding our knowledge of the primacy between AG and BG residues in the formation of stable SOM. These studies have left a gap in the current understanding in the role of AG and BG residues in the formation of stable SOM. Questions remain as to what extent the differences in AG and BG contributions to the stable C pool are driven by differences in biochemical quality.

Condensed tannins are oligomeric and polymeric flavonoids synthesized through the phenylpropanoid pathway of plants. Tannins represent a complex range of phenolic compounds that are present in many plant tissues and as such, have diverse physiological and ecological functions (Xie and Dixon, 2005). For example, tannins present in the leaves of many plants provide a means of preventing insect herbivory, while tannins in the seed coats may provide a function against fungal infection (Caldas and Blair 2009). Moreover, condensed tannins also comprise a large group of plant phenolic secondary metabolites which have been reported to alter rhizosphere soil microbial communities (Tilston et al., 2013), inhibit microbial enzyme activities important for nutrient cycling (Appel, 1993), reduce rates of N mineralization thereby inhibiting nitrification (Hättenschwiler and Vitousek, 2000) and alter decomposition of crop residues (Liebeke et al., 2015). Some genotypes of annual legume crops have been developed, through alteration of the phenylpropanoid pathway, to remove tannins from their seed coats. The removal or reduction of seed tannins through alteration of a biochemical pathway has the potential to change the tissue chemistry across the entire plant (Tilston et al., 2013), particularly lignin, flavanols and phenolic acids present in AG plant tissues, roots (Bazghaleh et al., 2018) and resulting rhizodeposits (Bazghaleh et al., 2018; Bekkara et al., 1998). However, to our knowledge, no information on decomposition or C dynamics of ZT lentils exists and the extent to which ZT lentils will affect soil microbial processing and CUE remain unclear.

Decomposition dynamics of AG and BG residues originating from ZT and TAN genotypes of lentil were studied in a laboratory incubation experiment. Genotypic variations allow for the observation of small-scale biochemical differences in decomposition and C dynamics of ZT lentil varieties, while the use of AG and BG residues enable us to elucidate not only biochemical quality on decomposition, but further clarify the role of root detritus in the formation and stability of the stable soil C pool. Laboratory incubations allowed for the direct analysis of residue biochemical quality on decomposition dynamics without environmental variability. With controlled environmental conditions and the homogenization of soil, soil microbial responses specific to tissue and genotype could be isolated. The hypotheses were that (1) BG residues and TAN

genotypes would respire at lower rates and release cumulatively less $CO_2$-C than AG residues and ZT genotypes respectively and (2) the resulting differences would be controlled by the soil microbial communities.

## 4.4. Materials and Methods

### 4.4.1. Seed selection

Two zero-tannin and two TAN lentil genotypes were used in this study. Zero-tannin one (LR-30-32) and TAN 1 (LR-30-101) belong to the lentil recombinant inbred line (RIL) population LR-30. This population was derived from a cross between the brown seed coat cultivar CDC Robin (genotype *Ggc Tgc Tan*) and a zero-tannin plant from the breeding line 2670b (genotype *Ggc Tgc tan*). Both genotypes are homozygous for *Tgc* and the resulting population have either normal brown (*Ggc Tgc Tan*) or gray zero-tannin (*Ggc Tgc tan*) seed coats based on segregation of the dominant or recessive alleles at the *Tan* locus (Mahla et al., 2016). Zero-tannin one and TAN 1 are therefore genetically related and were chosen to minimize additional genetic effects, allowing any differences found to be attributed to the expression of the *tan* gene. Conversely, ZT 2 (ZT4) and TAN 2 (cv. CDC Imvincible) do not share a common background, thus genotypic differences exceed those of the *tan* locus. Further, Bazghaleh et al. (2018) showed significant differences in the polyphenol content of root tissues in a comparison between ZT4 and a small green lentil variety similar to CDC Imvincible. It is important to note that ZT genotypes of lentil do contain minimal amounts of specific tannins and are not explicitly ‘zero’ in tannin content (Mirali et al. 2016; Bazghaleh et al., 2018).

### 4.4.2. Plant tissue preparation

Surface soil (0-15 cm), classified as a Dark Brown Chernozem (Bradwell association), was collected from the Goodale Research Farm in the winter of 2016. The soil was a fine sandy loam with a pH of 6.2. Prior to seeding, soil was air dried and sieved to 2 mm. Four replications of each genotype were grown in the greenhouse in a completely randomized design, at a density of 3 seeds per 8 inch pot. Plants were watered regularly with tap water and frequently rotated on the greenhouse bench to ensure homogenous plant illumination during growth. Both BG and AG plant components were harvested at crop maturity (84 d after seeding). Belowground tissues were

washed in tap water over a 2 mm sieve to remove soil. Seeds and chaff were removed from AG plant components and both BG and AG tissues were allowed to air dry prior to processing. Tissues were ground in a blender to approximately 2 mm in size and total C and N was assessed using dry combustion at 1100 $^{o}$C (TruMac elemental analyzer, Leco Corporation, Michigan, USA), (Table 4.1). Plant biological replicates were carried through and used as incubation replications.

**Table 4.1. Total carbon (C) and nitrogen (N) from above and belowground tissues of tannin (TAN) and zero-tannin (ZT) lentil genotypes, as determined by dry combustion at 1100 ºC.**

| Genotype | mg C $g^{-1}$ | mg N $g^{-1}$ | C:N | g C incubated | g N incubated |
|---|---|---|---|---|---|
| | | | Aboveground | | |
| TAN 1 | 451.50 ± 2.22ab† | 12.33 ± 0.57ab | 36.90 ± 1.95ab | 18.06 ± 0.09ab | 0.49 ± 0.02ab |
| TAN 2 | 451.25 ± 2.5ab | 10.24 ± 0.54a | 44.40 ± 2.10b | 18.05 ± 0.1ab | 0.41 ± 0.02a |
| ZT 1 | 449.25 ± 2.69a | 14.43 ± 1.09b | 31.68 ± 2.39a | 17.97 ± 0.11a | 0.58 ± 0.04b |
| ZT 2 | 461.00 ± 1.87b | 15.73 ± 0.94b | 29.65 ± 1.88a | 18.44 ± 0.07b | 0.63 ± 0.04b |
| | | | Belowground | | |
| TAN 1 | 378.00 ± 22.95b | 20.67 ± 1.53a | 18.34 ± 0.24b | 15.12 ± 0.92b | 0.83 ± 0.06a |
| TAN 2 | 268.75 ± 10.51a | 15.80 ± 0.53a | 17.01 ± 0.37b | 10.75 ± 0.42a | 0.63 ± 0.02a |
| ZT 1 | 285.50 ± 20.86ab | 18.95 ± 1.66a | 15.11 ± 0.22a | 11.42 ± 0.83ab | 0.76 ± 0.07a |
| ZT 2 | 390.00 ± ND‡ab | 23.90 ± NDa | 16.32 ± NDab | 15.60 ± NDab | 0.96 ± NDa |
| *P* value | | | | | |
| Tissue | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

†Mean values, n=4, (±SE) followed by similar letters within each tissue type are not significantly different at $P > 0.05$ based on Tukey's HSD.
‡No data available, n=1.

### 4.4.3. Microcosm preparation and $CO_2$ analysis

Soil was pre-incubated for 10 d at 32.5% water holding capacity (WHC) and 22 °C prior to microcosm preparation. Water holding capacity was determined by saturating the soils, allowing them to freely drain for 24 h and determining gravimetric water content after oven-drying at 105 °C. Four hundred mg of coarsely ground crop residue was mixed into 75 g of oven dried equivalent soil. The soil-residue mixture was then packed into microcosms (4.2 cm dia. by 9.2 cm height) at a bulk density of 1.2 g $cm^{-3}$. There were four replicate microcosms for each of nine treatments (4

genotypes x 2 tissue types + 1 no residue control), with four destructive sampling periods at 9, 29, 57 and 107 d for a total of 144 units. Soil microcosms were placed in 1 L mason jars fitted with a butyl rubber septum on the lid and incubated in the dark at 20 °C. One blank Mason jar was included for each replication. Microcosms were maintained at 65% WHC, by weight, throughout the 107 d incubation.

Gas efflux from soil microcosms was measured 1, 2, 4, 9, 15, 22, 29, 43, 57, 77, 91 and 107 d. after initiation of the incubation. On each sampling day, headspace gas samples were collected (20 mL) using a needle and syringe and immediately injected into an infrared gas analyzer to determine $CO_2$ concentration (LI-820, LICOR environmental, Lincoln, NE). After each sampling, the jars were flushed with $CO_2$-free air (1 L $min^{-1}$) for seven minutes and returned to the incubator.

### 4.4.4. Soil sampling and analysis.

Soil microcosms (n = 36) were destructively sampled at 9, 29, 57 and 107 d following the initiation of the incubation. Gravimetric moisture content was immediately determined on fresh soils (oven-dried at 105 °C). Soils from each microcosm were separated into three subsamples; one was freeze-dried and used for PLFA, the others stored at -20 °C for extracellular enzyme assays and microbial biomass determinations.

#### *4.4.4.1. Extracellular enzyme activities*

Activities of extracellular enzymes involved in the hydrolysis and oxidation of organic compounds important to soil C cycling were determined on thawed soils previously stored at -20 °C. The hydrolytic enzymes β-glucosidase (BGL) and cellobiohydrolase (CBH) were assayed based on the fluorometric determination of 4-methylumbelliferone (MUB) released from synthetic substrates (Bell et al., 2013). The oxidative PPO was assayed based on colorimetric determination of L-DOPA. The substrates used were 25 mM MUB-β-D-glucopyranoside, 25 mM MUB-β-D-cellobioside and 10 mM L-DOPA for determination of BGL, CBH and PPO respectively. Soil aliquots (1.0 g) were combined with 125 mL 50 mM sodium acetate buffer, pH 6.2, and homogenized in a blender for 1 min. The resulting suspensions were continuously stirred on a magnetic stir plate while 1.8 mL aliquots were dispensed into 5 mL centrifuge tubes (Hargreaves and Hofmockel, 2015). Assayed samples received 0.45 mL substrate. Blank tubes received 0.45 mL of acetate

buffer plus 1.8 mL of sample suspension. Negative controls received 0.45 mL substrate solution plus 1.8 mL of acetate buffer. Quench standards received 0.45 mL of standard plus 1.8 mL sample suspension. Reference standards received 0.45 mL of standard plus 1.8 mL acetate buffer. Quench standards were not used for PPO. Tubes were incubated in the dark while shaking at 140 rpm 21 °C for 2.5 h (BGL and CBH) or still for 20 h (PPO). The reactions were stopped by centrifuging at 4 °C. Fluorescence (excitation 360 nm, emission 465 nm) or absorbance (460 nm) in the supernatant was measured on a microplate reader (F5 Multi-Mode Microplate Reader, Molecular Devices, San Francisco, CA). Hydrolytic enzyme activities were determined using a standard curve of MUB with concentrations ranging between 0 and 100 μM. All enzymes activities are expressed in units of nmol substrate consumed $g^{-1}$ dry weight $h^{-1}$.

*4.4.4.2. Microbial PLFA extraction and analysis*

Phospholipid fatty acids were extracted from soil as a measure of viable microbial biomass and to determine microbial community composition. PLFAs were extracted as outlined by Helgason et al. (2010). Briefly, lipids were extracted from 4 g ground freeze-dried soil using a phosphate buffered solution. Phospholipids, neutral lipids and glycolipids were collected in the organic phase and fractioned using a solid phase extraction column (0.50 g Si; Varian Inc., Mississauga, ON). Phospholipids were subjected to mild alkaline methanolysis to produce FAMEs and analyzed by GC- FID (Scion 436-GC, Scion Instruments, Livingston, WL). Peaks were identified by comparing Kovats indices with retention times normalized to fatty acid standards and quantified based on the addition of internal standard methyl nonadecanoate (19:0). Fatty acids were separated into microbial groups according to Helgason et al. (2010). Total biomass was calculated by the sum of all identified peaks. Bacterial biomass was assessed using eighteen biomarkers (i14:0, i15:0, a15:0, 15:1ω6c, i16:0, 16:1ω9c, 16:1ω7c, i17:0, a17:0, cy17:0, i18:0, 18:1ω7c, 18:1ω9c, 18:1ω5c, cy19:0, 20:1ω9c, 10Me16:0, 10Me18:0). Bacteria were further separated into Gram+ (i14:0, i15:0, a15:0, i16:0, i17:0, a17:0, i18:0) and Gram- (15:1ω6c, 16:1ω9c, 16:1ω7c, cy17:0, 18:1ω9c, 18:1 ω7c, 18:1 ω5c, cy19:0, 20:1ω9c). Saprotrophic and AM fungi were identified using the 18:2ω6c and 16:1ω5c biomarkers respectively. Finally, biomarkers 10Me16:0 and 10Me18:0 were used as indicators of actinobacteria.

*4.4.4.3. Microbial biomass carbon*

Chloroform fumigation-extraction (Voroney et al., 2008) was used to determine total microbial biomass carbon (MBC). Two aliquots (5 g) of each soil were weighed separately into 50 mL glass jars to be used for fumigation and immediate extraction. Un-fumigated samples were subject to 20 mL 0.5 M $K_2SO_4$ and allowed to shake for 1 h at 200 rpm. After shaking, the suspensions were filtered through Whatman No. 42 filter. Fumigated samples were placed in desiccators containing ethanol free chloroform for 24 h prior to extraction. Blank jars were included for both un-fumigated and fumigated treatments and were treated in the same manner. Organic carbon in all filtrates was determined on a Shimadzu TOC-V organic carbon analyzer (Shimadzu, Columbia, MD) via wet oxidation. Chloroform labile C was calculated as the difference between C extracted from the chloroform fumigated and the non-fumigated sample. All results are expressed on an oven-dry basis. No conversion factor ($k_{EC}$) was used to convert chloroform labile C to MBC because of the $k_{EC}$ values cited in literature (0.41–0.58), none have been tested for use in the soil used here. Metabolic quotients were calculated as the ratio of cumulative respiration to MBC.

**4.4.5. Statistical analyses**

The effects of tissue type (AG *vs*. BG), genotype, and time on respiration rates of the full incubation were compared using a full factorial three-way ANOVA. A day by tissue and day by genotype interaction were observed. In order to observe treatment effects on respiration rates while minimizing interactions, data was subset (until day 9 for genotype and day 29 for tissue type). The effect of genotype ($p=0.015$) and tannin content ($p=0.007$) were individually assessed using a linear mixed model with tissue as a random factor and means were compared using Tukey's HSD post-hoc test. The effects of tissue type were compared using a Welch Two Sample t-test ($p<0.001$). Time, tissue type and their interaction effects on PLFA abundance, enzyme activity, metabolic quotients, MBC and extractable organic carbon (EOC) were compared using a full factorial two-way ANOVA. Genotype and tannin effects were compared individually using a linear mixed model with the lme function in the nlme package in R. Tissue type was used as a random factor. Community structure analysis was performed using non-metric multidimensional scaling of Bray-Curtis dissimilarities on log(mol%+1) transformed PLFA data. Significance testing was performed using permutational analysis of variance using the ADONIS function in the

Vegan 2.5-3 package (Oksanen et al., 2018) in R. Apart from community structure data, all data met ANOVA assumptions of normality and homogeneity of variance according to Shapiro Wilk and Levene's test, respectively. All statistical analyses were performed using R 3.5.1 (R Development Core Team, 2018).

## 4.5. Results

### 4.5.1. Soil and residue derived respiration

All residue-amended soils rapidly increased soil $CO_2$ efflux when compared to control soils. Generally, respiration rates were highest at the onset of decomposition and declined over time (Fig. 4.1A). Respiration rates for BG tissues were significantly lower than AG tissues ($P < 0.001$). Further, ZT residue amended soils respired at higher rates than TAN residue amended soils in both AG ($P$=0.002) and BG soils ($P$=0.018), though respiration rates for all soils began to converge by day nine (Fig. 4.1A). Though not significant, AG soils containing TAN genotypes respired less cumulative $CO_2$-C than soils containing ZT genotypes. In soils containing BG residues, ZT 2 and TAN 2 respired the most and least $CO_2$-C respectively; while cumulative $CO_2$-C respired was negligible between ZT 1 and TAN 1 respired (Fig. 4.1B). Averaged across genotypes, cumulative $CO_2$-C was 73% higher from soils amended with AG tissues compared to BG residues and 102.4% higher than control soils. Soils amended with BG residues respired 29.5% more $CO_2$-C than control soils.

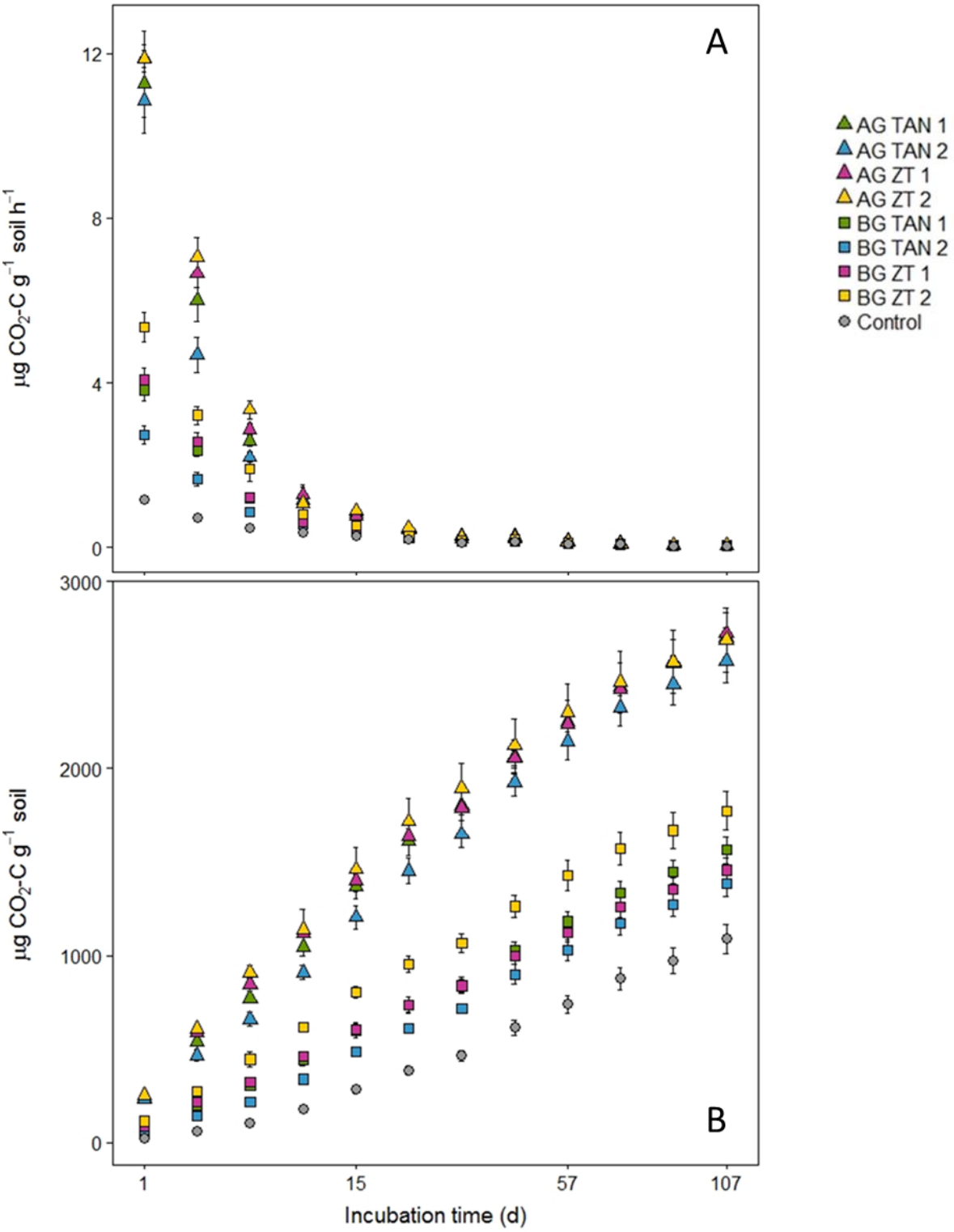


**Fig. 4.1. Total respiration rates (A) and cumulative $CO_2$-C (B) respired over a 107 d incubation in control and residue-amended soil microcosms containing residues from above (AG) and belowground (BG) tissues of tannin (TAN) and zero-tannin (ZT) lentil genotypes. Markers represent means and bars are standard errors of replicate samples (n = 4).**

### 4.5.2. Total microbial biomass carbon, extractable organic carbon and microbial metabolic quotients

Total MBC at the onset of decomposition in BG soils was 132% higher than control soils and remained relatively constant throughout the incubation, though a slight and non-significant decline was observed on day 29. Total microbial biomass in AG soils was 170% higher than in controls soils at the onset of decomposition and was followed by a significant decline ($P$=0.0221). Neither MBC nor EOC differed significantly by the end of the incubation. Metabolic quotients were significantly influenced by tissue type ($P$=0.04), with controls soils being the lowest, followed by BG soils. Genotype did not influence MBC, EOC nor microbial metabolic quotients.

**Table 4.2. Microbial biomass carbon (MBC), total extractable organic carbon (EOC) and metabolic quotients from residue amended and control soil microcosms over a 107 day incubation.**

| Incubation time (d) | MBC µg g soil $^{-1}$ | EOC µg g soil $^{-1}$ | Metabolic Quotient µg $CO_2$-C µg $^{-1}$ MBC |
|---|---|---|---|
| | | Aboveground | |
| 9 | 224.11 ± 4.83† | 395.12 ± 4.55 | 4.82 ± 0.16 |
| 29 | 173.14 ± 7.72 | 392.65 ± 6.70 | 10.84 ± 0.69 |
| 57 | 200.12 ± 12.65 | 338.09 ± 11.11 | 11.72 ± 0.69 |
| 107 | 180.95 ± 17.91 | 317.50 ± 12.89 | 19.62 ± 4.04 |
| | | Belowground | |
| 9 | 173.98 ± 4.98 | 318.28 ± 5.34 | 2.70 ± 0.17 |
| 29 | 165.23 ± 12.64 | 341.01 ± 4.55 | 5.37 ± 0.34 |
| 57 | 176.42 ± 8.76 | 305.18 ± 8.43 | 6.93 ± 0.37 |
| 107 | 176.57 ± 15.27 | 294.89 ± 17.05 | 9.59 ± 0.79 |
| | | Control | |
| 9 | 131.28 ± 10.05 | 272.8 ± 4.79 | 1.47 ± 0.13 |
| 29 | 112.86 ± 4.62 | 261.04 ± 5.23 | 4.26 ± 0.30 |
| 57 | 139.70 ± 13.49 | 258.96 ± 6.56 | 6.44 ± 1.04 |
| 107 | 108.99 ± 12.20 | 238.83 ± 5.65 | 12.12 ± 1.61 |

†Mean values (±SE) obtained from cumulative respiration and chloroform fumigation-extraction and are averaged across genotypes, n=16.

### 4.5.3. Abundance of PLFA biomarkers

In both control and residue amended soils, total PLFA abundance and the abundance of individual microbial groups significantly decreased with time ($P$< 0.001). While the abundance of

total biomass, total bacteria, fungi and Gram- bacteria were highest at the onset of decomposition and gradually declined as decomposition progressed, Gram+ bacteria, AMF and actinobacteria peaked on day 9 before declining (Fig. 4.2). Biomass differed significantly based on the nature of residue added ($P$<0.001). Soils incubated with AG lentil residues contained higher PLFA biomarkers than soils amended with lentil root residues, both being significantly higher than controls soils until day 57 of the incubation. The abundance of PLFA biomarkers was not affected by lentil genotype in residue-amended soils.

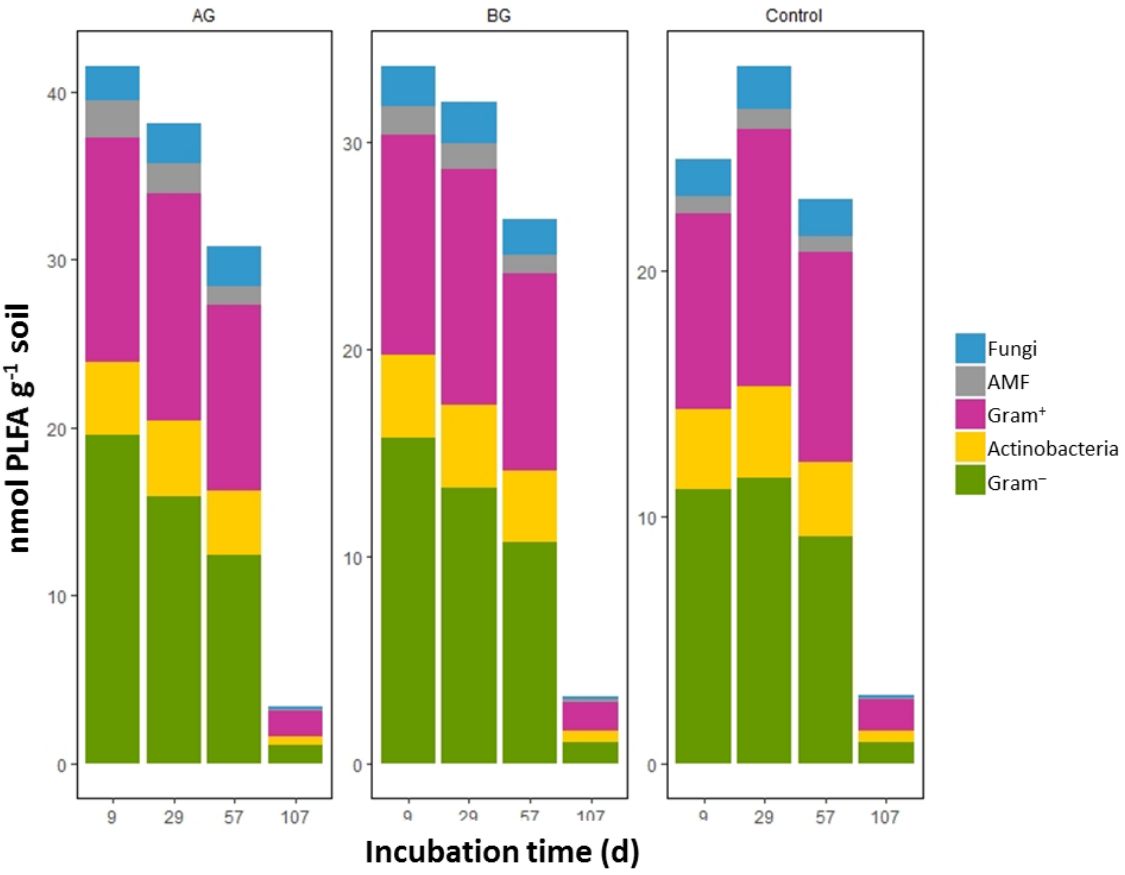


**Fig. 4.2. Total PLFA biomass and abundance within individual microbial groups over a 107 d incubation in control and residue-amended soil microcosms containing residues from aboveground (AG) and belowground (BG) tissues of tannin (TAN) and zero-tannin (ZT) lentil genotypes, n=4.**

### 4.5.4. Microbial community structure

Unconstrained ordination by NMDS of Bray–Curtis dissimilarity followed by ADONIS revealed significant differences in microbial community structure by time and tissue type, but not

genotype (time, $P$=0.001; tissue, $P$=0.001; genotype, $P$=0.312). Time points one and four separated very clearly from each other and from day two and three, while slight overlaps occurred between time points two and three. At each time point, AG, BG and control soils each clustered together, though these clusters became less distinct as time progressed (Fig. 4.4). The fungal to bacterial ratio (F:B) was affected by both time and tissue type (time, $P$<0.001; tissue, $P$=0.005) and was highest in AG soils and decreased with time. The Gram+ to Gram- ratio (GP:GN) was not affected by tissue or genotype, though increased with time ($P$<0.001). Averaged across tissue type, Gram- bacteria were more prominent at time point one, AMF at time point three and both Gram+ and actinobacteria at time point four. When each time point was analyzed separately, specific effects of tissue type became apparent (Figs. 4.4, 4.5, 4.6 and 4.7). While actinobacteria and AMF dominated in control soils at the beginning of decomposition, there was an abrupt change in composition by time point two, where AMF became one of the dominant groups in AG soils for the remainder of the decomposition. Fungi remained a dominant group in AG soils for the entirety of the incubation, while Gram+ and actinobacteria remained linked with control and BG soils.

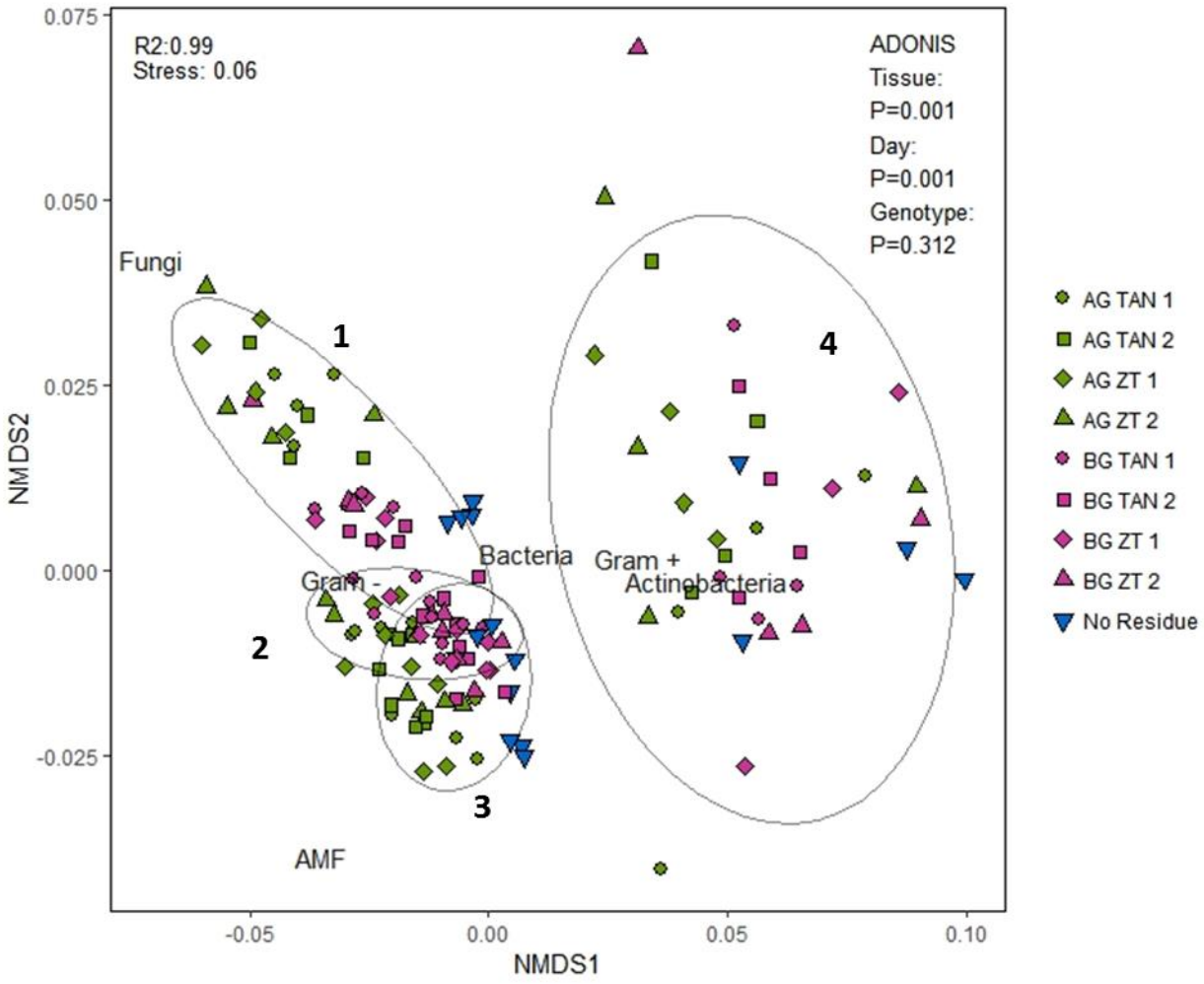


**Fig. 4.3. Non-metric multidimensional scaling of Bray–Curtis dissimilarity indicating PLFA profiles over a 107 d incubation in control and residue-amended soil microcosms containing residues from above (AG) and belowground (BG) tissues of tannin (TAN) and zero-tannin (ZT) lentil genotypes. Ellipses represent decomposition time points. Time points 1, 2, 3, and 4 represent incubation day 9, 29, 57 and 107 respectively, n=4.**

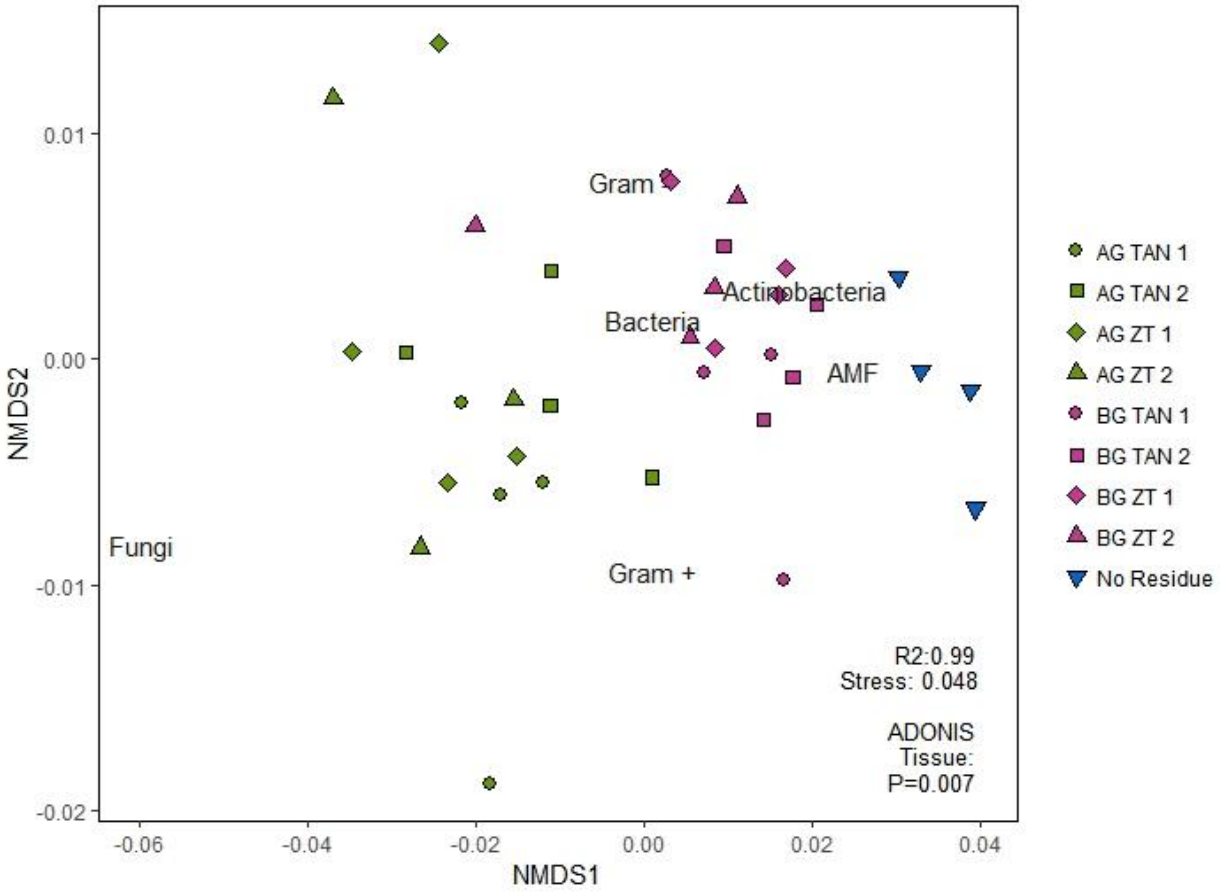


**Fig. 4.4. Non-metric multidimensional scaling of Bray–Curtis dissimilarity indicating PLFA profiles on day 9 of a 107 d incubation in control and residue-amended soil microcosms containing residues from above (AG) and belowground (BG) tissues of tannin (TAN) and zero-tannin (ZT) lentil genotypes, n=4.**

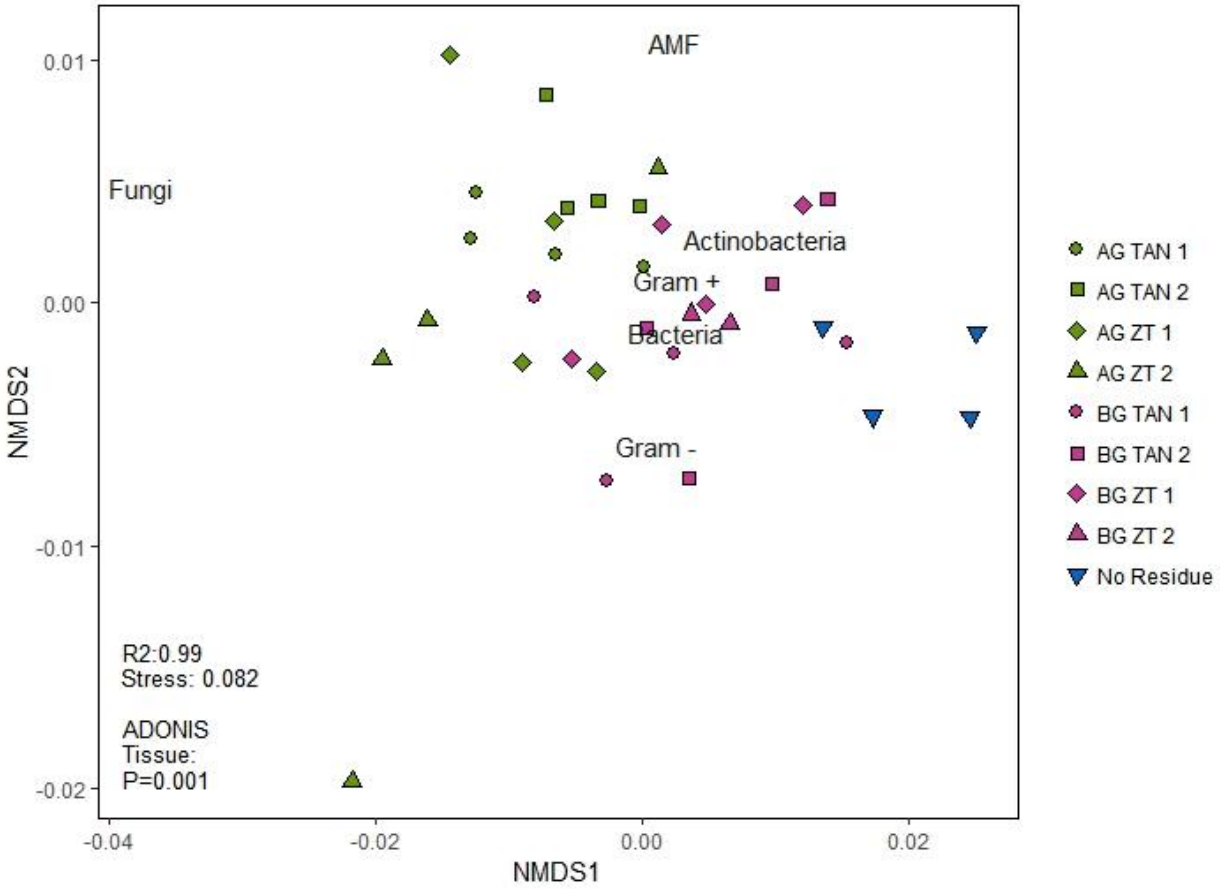


**Fig. 4.5. Non-metric multidimensional scaling of Bray–Curtis dissimilarity indicating PLFA profiles on day 29 of a 107 d incubation in control and residue-amended soil microcosms containing residues from above (AG) and belowground (BG) tissues of tannin (TAN) and zero-tannin (ZT) lentil genotypes, n=4.**

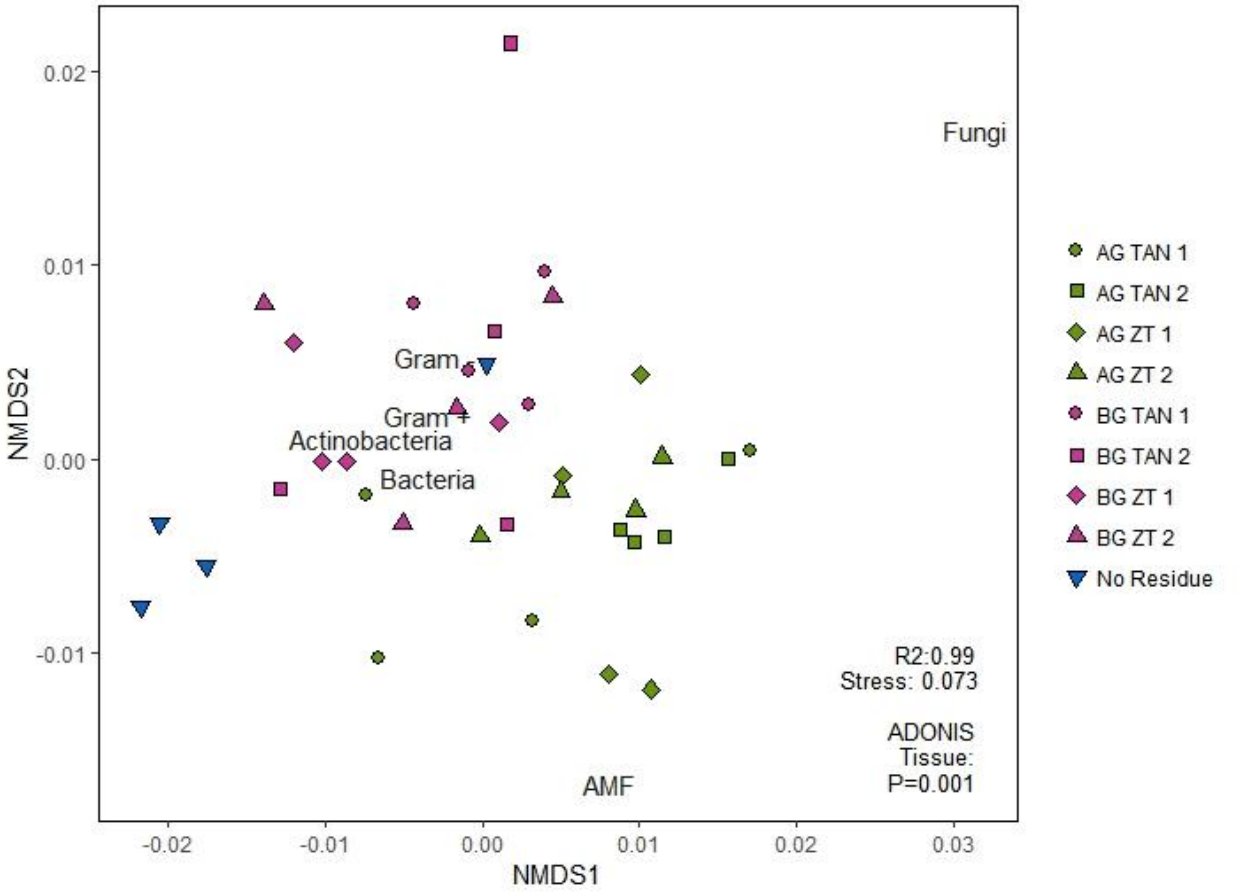


**Fig. 4.6. Non-metric multidimensional scaling of Bray–Curtis dissimilarity indicating PLFA profiles on day 57 of a 107 d incubation in control and residue-amended soil microcosms containing residues from above (AG) and belowground (BG) tissues of tannin (TAN) and zero-tannin (ZT) lentil genotypes, n=4.**

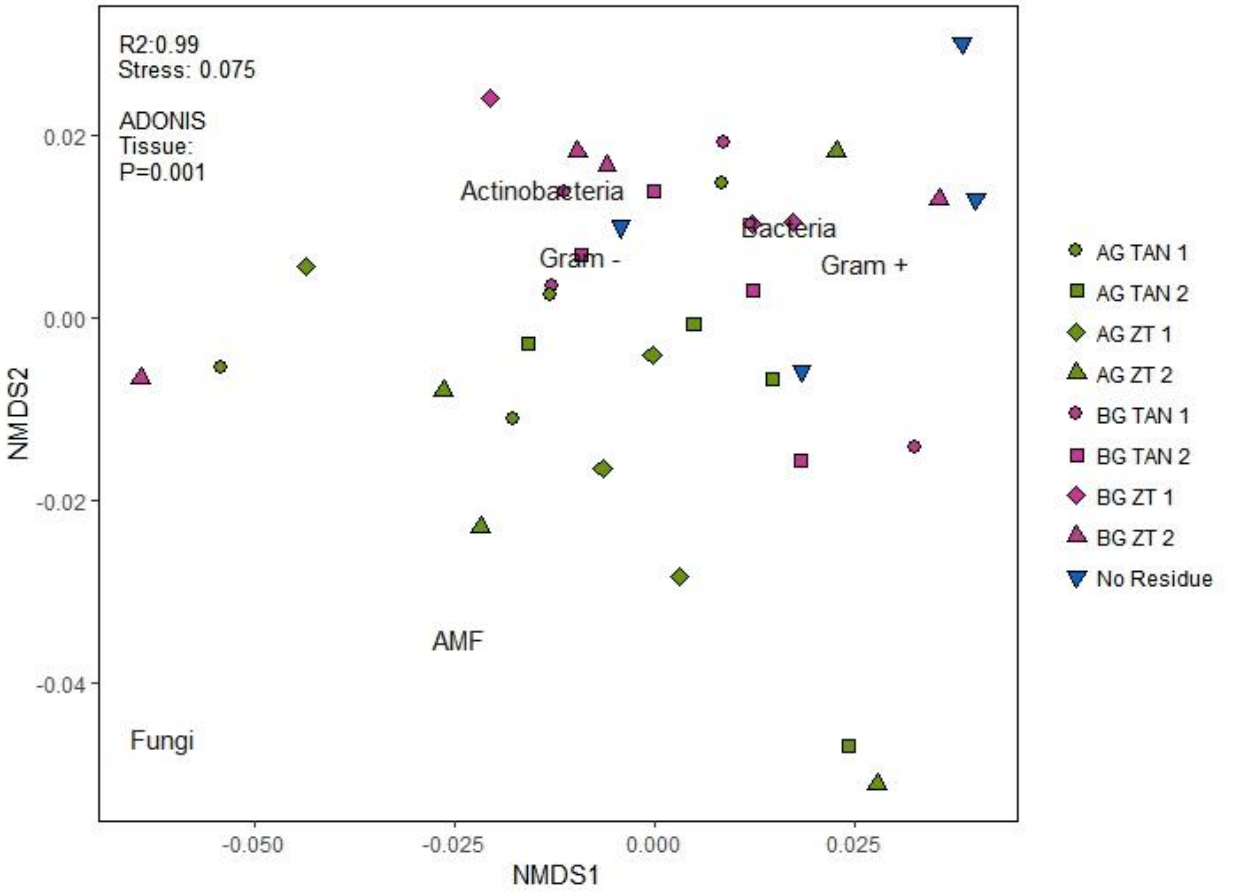


**Fig. 4.7. Non-metric multidimensional scaling of Bray–Curtis dissimilarity indicating PLFA profiles on day 107 of a 107 d incubation in control and residue-amended soil microcosms containing residues from above (AG) and belowground (BG) tissues of tannin (TAN) and zero-tannin (ZT) lentil genotypes, n=4.**

### 4.5.5. Extracellular enzyme activity

Extracellular enzyme activity differed significantly with time ($P$<0.001) in both control and residue amended soils. Enzyme activity was consistently lowest at the onset of decomposition, with CBH (Table 4.3) and BGL (Table 4.4) peaking on day 29 and PPO (Table 4.5) activity peaking on day 57. All enzyme activity declined as decomposition progressed. Cellobiohydrolase activity was significantly affected by tissue and genotype (tissue, $P$=0.033; genotype $P$=0.008) and the specific treatments driving these differences were AG ZT 2 and BG TAN 2.Neither β-

Glucosidase nor PPO were affected by genotype or tissue type though generally, extracellular enzyme activity was highest in control soils, followed by BG soils.

**Table 4.3. Cellobiohydrolase activity (nmol $h^{-1}$ $g^{-1}$ soil) over a 107 d incubation in control and residue-amended soil microcosms containing residues from above and below ground tissues of tannin (TAN) and zero-tannin (ZT) lentil genotypes.**

| Genotype | Incubation time (d) | | | |
|---|---|---|---|---|
| | 9 | 29 | 57 | 107 |
| | | | Aboveground | |
| TAN 1 | 115.06 ± 11.42† | 141.89 ± 5.88 | 173.63 ± 11.8 | 119.89 ± 8.10 |
| TAN 2 | 125.30 ± 16.64 | 183.08 ± 15.29 | 158.66 ± 5.82 | 148.20 ± 18.68 |
| ZT 1 | 69.20 ± 8.62 | 154.86 ± 9.87 | 169.42 ± 10.35 | 128.17 ± 12.17 |
| ZT 2 | 115.18 ± 16.48 | 179.33 ± 12.82 | 163.31 ± 19.37 | 142.04 ± 10.48 |
| | | | Belowground | |
| TAN 1 | 128.19 ± 6.1 | 163.34 ± 4.99 | 162.27 ± 20.54 | 135.26 ± 10.14 |
| TAN 2 | 127.59 ± 3.82 | 171.43 ± 7.39 | 160.62 ± 10.22 | 142.61 ± 10.81 |
| ZT 1 | 134.05 ± 14.95 | 177.53 ± 5.78 | 129.32 ± 4.23 | 142.21 ± 7.56 |
| ZT 2 | 130.99 ± 10.91 | 214.11 ± 32.76 | 189.38 ± 20.92 | 142.53 ± 9.76 |
| | | | Control | |
| | 130.07 ± 6.11 | 173.61 ± 16.53 | 145.57 ± 14.56 | 147.01 ± 28.32 |

†Mean values are followed by ±SE, n=4.

**Table 4.4. β-Glucosidase activity (nmol $h^{-1}$ $g^{-1}$ soil) over a 107 d incubation in control and residue-amended soil microcosms containing residues from above and below ground tissues of tannin (TAN) and zero-tannin (ZT) lentil genotypes.**

| Genotype | Incubation time (d) | | | |
|---|---|---|---|---|
| | 9 | 29 | 57 | 107 |
| | | | Aboveground | |
| TAN 1 | 545.16 ± 34.73† | 702.48 ± 53.47 | 722.73 ± 45.58 | 553.21 ± 41.35 |
| TAN 2 | 584.96 ± 11.50 | 814.65 ± 23.26 | 620.25 ± 19.73 | 615.57 ± 85.14 |
| ZT 1 | 466.78 ± 32.07 | 728.49 ± 25.05 | 668.46 ± 39.14 | 562.26 ± 77.28 |
| ZT 2 | 544.97 ± 37.65 | 742.14 ± 21.02 | 643.08 ± 17.23 | 579.67 ± 26.12 |
| | | | Belowground | |
| TAN 1 | 590.31 ± 11.46 | 698.52 ± 31.56 | 709.77 ± 77.55 | 546.66 ± 16.12 |
| TAN 2 | 552.49 ± 20.09 | 667.84 ± 8.07 | 668.99 ± 23.17 | 620.72 ± 46.68 |
| ZT 1 | 590.76 ± 7.40 | 725.35 ± 38.08 | 635.46 ± 25.87 | 605.34 ± 62.77 |
| ZT 2 | 640.04 ± 30.77 | 772.73 ± 34.88 | 702.67 ± 32.19 | 732.15 ± 100.57 |
| | | | Control | |
| | 599.53 ± 22.35 | 765.68 ± 10.74 | 656.08 ± 26.97 | 628.01 ± 95.51 |

| *P* Value‡ | |
|---|---|
| Time | < 0.001 |
| Tissue | 0.138 |
| Genotype | 0.197 |

†Mean values are followed by ±SE, n=4.

**Table 4.5. Polyphenol Oxidase activity (nmol $h^{-1}$ $g^{-1}$ soil) over a 107 d incubation in control and residue-amended soil microcosms containing residues from above and below ground tissues of tannin (TAN) and zero-tannin (ZT) lentil genotypes.**

| Genotype | Incubation time (d) | | | |
|---|---|---|---|---|
| | 9 | 29 | 57 | 107 |
| | | | Aboveground | |
| TAN 1 | 54.44 ± 3.79† | 62.08 ± 2.79 | 62.62 ± 12.54 | 62.84 ± 8.79 |
| TAN 2 | 45.38 ± 1.68 | 57.44 ± 3.13 | 75.65 ± 10.02 | 60.64 ± 11.33 |
| ZT 1 | 51.20 ± 4.18 | 61.95 ± 5.15 | 67.10 ± 7.88 | 65.29 ± 15.56 |
| ZT 2 | 51.80 ± 3.02 | 68.33 ± 3.40 | 69.56 ± 6.97 | 51.55 ± 6.59 |
| | | | Belowground | |
| TAN 1 | 52.89 ± 5.87 | 56.12 ± 4.20 | 86.56 ± 20.68 | 47.3 ± 8.89 |
| TAN 2 | 49.63 ± 4.36 | 59.62 ± 5.84 | 71.85 ± 5.17 | 71.22 ± 7.94 |
| ZT 1 | 55.63 ± 6.94 | 66.43 ± 3.44 | 71.39 ± 6.26 | 61.94 ± 16.68 |
| ZT 2 | 56.57 ± 7.39 | 69.19 ± 11.83 | 73.64 ± 3.28 | 63.74 ± 5.85 |
| | | | Control | |
| | 57.12 ± 6.64 | 62.99 ± 4.86 | 63.14 ± 1.69 | 66.69 ± 17.1 |

†Mean values are followed by ±SE, n=4.

## 4.6. Discussion

Soil microbial CUE is the relative partitioning between microbial anabolic and catabolic processes; this partitioning plays a critical role in C sequestration, nutrient availability and SOM formation, thus controlling the flow of C through terrestrial ecosystems. Less $CO_2$-C was respired per unit MBC with BG residues compared to microcosms incubated with AG residues. Moreover, soil microbial community structure was significantly different between soils incubated with AG and BG residues, indicating that biochemically complex compounds have the potential to be degraded with a better efficiency than what has been previously thought and that this difference is mediated by the soil microbial communities.

Crop residues vary in their structural and chemical composition (Adair et al. 2008). Residues are often classified on the basis of carbon to nitrogen ratios (C:N) and the quantity of complex compounds such as phenols, tannins, or lignin (Stewart et al. 2015; Wang et al. 2015). The typical C:N of AG lentil residues is 36 (Bremer et al., 1991) and between 27 and 30 for BG residues (Gan et al., 2011). Though C:N values for AG residues used in this study were consistent with the literature, values for BG residues were remarkably low. Two explanations may be

possible. Firstly, the inclusion of root nodules in overall BG residue composition may have contributed to the above normal N values (Gan et al., 2011) observed in BG residues. Secondly, C values were slightly lower than expected (Bremer et al., 1991) and can potentially be attributed to the presence of rhizosphere soil attached to roots subsequent to washing. The increased N and decreased C values thus shifted C:N values in a linear direction towards less C and more N. Although polyphenol characterization was not conducted, a prior study (Bazghaleh et al., 2018) indicated that root tissues of TAN 2 and ZT 2 are significantly different in the composition of their phenolic compounds. Further, phenotypic differences in AG plant components between TAN and ZT lentil genotypes can be observed (Fig. 4.7).

Our first hypothesis assumed different mineralization rates between lentil genotypes and litter input types. In accordance, soils incubated with AG residues respired faster and proportionally more $CO_2$-C per g of residue than soils incubated with BG residues. Our findings are supported by other studies (Birouste et al., 2012; Prescott, 2010; Silver and Maya 2001) and are generally explained by the biochemical composition of roots, which contain relatively less readily decomposable compounds and higher amounts of complex compounds such as lignin, suberin, phenols and tannin than AG residues (Aber and Melillo, 1982; Lian et al., 2016). Other characteristics, such as pigmentation, have also been reported to influence decomposition (Fan and Guo, 2010; Goebel et al., 2011). Zero-tannin lentil genotypes contain less polyphenols in their structural components, as visualized by the lack of anthocyanin pigmentation in their stems (Fig. 4.7). Accordingly, ZT genotypes respired more cumulative $CO_2$-C initially than tannin genotypes in both AG and BG tissues. Priming represents the changes in native SOM as a result of exogenous substrate inputs (Jenkinson et al., 1985; Kuzyakov 2010). When soil microorganisms are C-limited, fresh inputs may alter their activities such that a priming effect is elicited (Blagodatskaya and Kuzyakov 2008). Priming has been found to be inversely proportional to the intensive phase (first 3 weeks) of decomposition (Shabaaz et al. 2017) and potentially explains why initial differences in $CO_2$ efflux observed between ZT and TAN genotypes are no longer seen after day 22 of the incubation. As resources become limited, soil and microbially derived C begins to get recycled. Interestingly, upon completion of the incubation ZT 2 and TAN 2 soils had respired cumulatively the most and the least $CO_2$-C respectively, while TAN 1 and ZT 1 were nearly indistinguishable from each other. Therefore, potential differences induced by the presence or absence of the *tan* gene resulted in negligible differences in respiration.

**Fig. 4.7. Zero-tannin (ZT) and tannin (TAN) containing genotypes of lentil plants used as residues for this incubation study. Zero-tannin genotypes have green stems and TAN genotypes have reddish-brown stems.**

While it was found that BG residues mineralize at lower rates and result in lower $CO_2$-C respiration fluxes than AG residues, these results need to be related to the portion of substrate-C retained in the soil, either within the microbial biomass or via stabilizations within the soil matrix. However, more complex methods such as the use of stable isotopes would be required in order to do so (Cotrufo et al., 2015). Microbial CUE refers to the relative partitioning of substrate-C between microbial anabolic and catabolic processes. Total MBC at the onset of decomposition in BG soils was 132% higher than in control soils and remained relatively constant throughout the incubation, though a slight and non- significant decline was observed on day 29. Total MBC in AG soils was 170% higher than in control soils at the onset of decomposition and then decline rapidly. The fast decline of MBC at day 29 demonstrates the exhaustion of the labile portion of residue derived C, a result also noted in other studies (Blagodatskaya et al. 2011b; Wang et al.

2016). This behavior mimics that of r-strategists, who grow rapidly on easily available substrate and have high rates of turn over (Bastian et al., 2009; Pascault et al., 2013). Further, Fontaine et al (2003) proposed that a priming effect results from the succession of r- to k-strategists. Moreover, the increase of extracellular enzyme activities after the intensive phase of residue decomposition confirms that microorganisms were at a nutrient limitation, potentially eliciting a priming response (Blagodatskaya et al., 2014). This priming response can be further observed by the acceleration of biomass production following the sharp decline. Microbial abundance, measured by PLFA, further displays a greater increase in biomass for AG soils than BG soils. Indeed, PLFA is a measure of viable microbial biomass, thus patterns of decrease and increase associated with AG soils are not observable through this method.

Recent studies have suggested that simple C forms will decompose at a faster rate initially, but store more C in the soil via microbial residues (Cotrufo et al., 2013). Total MBC and total EOC between AG and BG were not significantly different at the end of the incubation. Though MBC and EOC may not capture all microbial byproducts, these results may suggest that biochemically complex compounds have the potential to be stabilized within the soil matrix through microbial byproducts to the same extent as labile residues. The microbial metabolic quotient ($qCO_2$) for each tissue and genotype was calculated for each tissue and genotype, as it has been used as a proxy for microbial CUE (Bailey et al., 2018). Root tissues contain significantly more structurally complex compounds than AG tissues. Therefore, either a lower CUE or a higher metabolic quotient for these tissues would be expected. While this hypothesis has been tested and confirmed in the laboratory (Córdova et al., 2018; Haddix et al., 2016; Lavallee et al., 2018), results of the current study did not corroborate. The lower $qCO_2$ observed in soils containing BG residues suggest that soil microorganisms in these soils likely produced more cell mass per unit of C respired thus processed root residues with a higher efficiency than AG residues. Extracellular enzyme activities were inversely proportional to MBC in both AG and BG soils, thus indicating the relatively complexity between AG and BG residues and further confirming the processing efficiency of microorganisms in BG soils.

Our second hypothesis assumed a shift in decomposer community structure between ZT and TAN lentil genotypes and between AG and BG residues. Results confirmed, at least in part, our hypothesis. Soil microorganisms contribute to the processing and stabilization of C inputs from plant litter, root exudates and microbial turnover (Liang et al., 2017). Typically, Gram- bacteria

are associated with the consumption of easily degradable substrates (Treonis et al., 2004; Creamer et al., 2016), while Gram+ bacteria appear to be more important in the decomposition of SOM (Kramer and Gleixner, 2006, 2008). Moreover, experiments have identified saprotrophic fungi as major players in the rapid processing of easily available C (Pausch et al., 2016). Our results support these findings. Bacteria and saprotrophic fungi dominated soils incubated with AG residues rather than BG residues. Indeed, AG residues may not be considered easily available though, when compared with the structural components of root tissues, might be considered *more* easily available. Gram+, predominantly actinobacteria, tended to associate in soils incubated with BG residues and with control soils which did not receive residue. Certainly, BG soils contain more structural components than AG soils and could potentially explain the association with Gram+ and actinobacteria. However, I acknowledge that root residues may not be completely soil-free upon addition to soil microcosms, thus causing them to behave similarly to control soils. Nevertheless, the GP:GN increased throughout the course of the incubation. As the availability of readily decomposable compounds decrease with time, it is expected to see proportionate shifts in the decomposer communities. All microcosms were incubated with equivalent soil and therefore decomposition in all microcosms was limited to the same intrinsic microbial communities, thus shifts in these communities are likely dependent on the quality of available substrate. It has been hypothesized that microorganisms are more sensitive to changes in macromolecular C (i.e. lignin and tannin) than simple C (i.e. sugars and amino acids) (Schimel and Gulledge 1998). In agreement, this study showed that genotype had no influence on the structure of the microbial communities, indicating either that 1) minor differences in tissue quality do not affect microbial processing of such inputs or 2) C:N are more important in the regulation of decomposition dynamics than C stoichiometry.

Arbuscular mycorrhizal fungi predominantly form symbiotic relations with plant root systems (Hassan and Mathesius, 2012), potentially limiting their ability to produce extracellular enzymes. Accordingly, AMF persistence should be proportionally related to the availability of nutrients. Surprisingly, AMF were more prevalent in control soils at the onset of decomposition. This phenomenon did not persist, however, and for the remainder of the incubation AMF was observed to be dominant in soils containing AG residues. Due to their sessile lifestyle and need to adapt to variable environments, plants have evolved remarkable plasticity in their growth and morphology BG (Fitter 1994; de Kroon and Hutchings 1995; Palmer et al. 2012), enabling them

to span large areas of soil. It is likely that the initial occurrence of AMF in control soils was a result of the carryover of the AMF naturally existing in fine roots present in the soil used for the decomposition, and not due to its preference in the degradation of complex C compounds.

### 4.7. Conclusion

This study examined the decomposition dynamics of tissues varying in their biochemical quality by comparing AG and BG tissues originating from ZT and TAN genotypes of lentil. Soil microbial communities in microcosms incubated with BG residues produced proportionately more cell biomass per unit of C degraded than microcosms incubated with AG soils. Phospholipid fatty acid profiles were significantly different between soils incubated with AG and BG residues. While BG and control soils were dominated primarily by oligotrophic organisms, AG soils were dominated mainly by copiotrophic microorganisms, indicating the relative complexity between AG and BG lentil tissues. Furthermore, extracellular enzyme activities were found to be the highest in control soils, followed by BG soils, indicating that BG and control soils contained less readily decomposable compounds than AG soils. Although previous findings indicate that simple C forms would respire faster than complex residues but store proportionately more C in the soil via microbial byproducts and necromass, our results indicate that biochemically complex compounds have the potential to be degraded with a better efficiency than what has been previously thought and that this difference is mediated by the soil microbial communities. Furthermore, most studies that have found greater retention of root tissues in the soil, did not compare the decomposition of root and shoot tissues equally on a per C or per g basis. While this may be realistic, it does not give an accurate comparison of the stability between root and shoot tissues in the soil. Lastly, it should be acknowledged that metabolic quotients are an indirect method for studying microbial C use efficiency. Hence, our investigation is not a final point, but rather an interesting perspective for future studies, which could combine the use of $^{13}C$ stable isotope probing with laboratory facilitated decomposition of root tissues in a range of plant species. By these means, linking of microbial community structure and function with BG processes will be approached without incorporating environmental variabilities.

# 5. SYNTHESIS AND CONCLUSIONS

The majority of soil organic carbon (SOC) results both directly from aboveground (AG) and belowground (BG) plant structural components, rhizodeposits and leaf litter leachate, and indirectly by the transfer of carbon (C) enriched compounds from roots to soil microbes (Cotrufo et al., 2013). The processing of organic C is primarily mediated by soil microorganisms. By partitioning C through anabolic and catabolic processes, soil microorganisms control the flow of C through terrestrial ecosystems. As microorganisms metabolize organic compounds as a need to satisfy heterotrophic demands for C and energy, C partitioning should be related to both the physiology of the active microbial population and the biochemical quality of C substrate. Because SOC is initially derived from photosynthetically captured C, the composition of plant C inputs depends on genetic factors and varies widely among plant species (Badri and Vivanco, 2009). Zero-tannin (ZT) lentils have been selectively bred, through alteration of the phenylpropanoid pathway, to remove tannins from their seed coats. Any alterations in a biochemical pathway of a plant has the potential to alter the tissue chemistry across the entire plant, including root tissues and rhizodeposits (Tilston et al., 2013); and any changes in the biotic processing of C inputs has the potential to shift pulse-crop derived C trajectories through the soil. Indeed, countless studies have investigated soil microbial communities under varying crop and treatment combinations however, no studies have directly assessed the effects of ZT lentils on rhizosphere microbial communities. Moreover, the contribution of chemically complex C compounds to stable soil carbon pools is currently under debate. For many decades, it was assumed that decomposition rates are inversely proportional to the quantity of substrate C stabilized in the soil. For example, more complex substrate would hinder microbial decomposition, leading to larger quantities of C remaining in the soil. Contradicting this hypothesis, many authors (Cotrufo et al., 2013; Lehmann and Kleber, 2015; Kallenbach et al., 2016) now propose that higher quality substrate may still be decomposed at higher rates, though more C will be retained in the soil through microbial necromass and byproducts. The objective of this research was to examine the effects of ZT lentil genotypes on soil microbial communities and carbon cycling processes, both during plant growth

and after, when compared to the traditional tannin (TAN) genotypes and to broaden the current understanding of soil carbon stability and microbial carbon use efficiency by investigating soil decomposition between AG and BG plant tissues.

### 5.1. Summary of Findings

Both substrate quality and microbial community composition are important factors governing microbial CUE (Manzoni et al., 2012; Sinsabaugh et al., 2013). Microbial community composition can affect CUE as a result of varied life strategies (Fierer et al., 2007). For example, copiotrophic microorganisms thrive in nutrient rich environments and are characterized by high growth rates and fast turnover. By contrast, oligotrophs are characterized by their ability to grow under low substrate concentrations, and generally possess a higher substrate utilization efficiency (Fierer et al., 2007; Roller and Schmidt, 2015). In Chapter 3, utilizing a $^{13}C$ pulse labelling technique, I found that the quantity of rhizodeposition (%CdfR) did not significantly differ between ZT and TAN genotypes. Further, the amount of $^{13}C$ incorporated in the total microbial biomass of microorganisms under TAN genotypes was significantly higher than that of ZT genotypes, indicating that microorganisms in TAN soils are preferentially utilizing root derived C. Microbial community composition differed significantly in the rhizosphere between ZT and TAN genotypes. While ZT soils were primarily dominated by oligotrophic microorganisms (i.e., Gram+ bacteria), TAN soils were dominated mainly by copiotrophic microorganisms (i.e., Gram- bacteria and fungi). Moreover, though %CdfR did not significantly differ between TAN and ZT genotypes, total microbial abundance was significantly higher in TAN soils. Due to the ability of oligotrophic microorganisms to utilize complex C forms, these results may suggest the susceptibility of ZT soils to SOM priming, thus resulting in a net C loss to the atmosphere. In contrast, the higher microbial abundance and incorporation of root derived C into rhizosphere microorganisms under TAN genotypes likely indicates the potential for increased soil C retention through microbial necromass and byproducts.

Interestingly, respiration rates and cumulative C loss did not significantly differ between TAN and ZT soils (Chapter 4). The rhizosphere is highly dynamic and supports a dense microbial population which is more diverse, active, and synergistic than in non-rhizosphere soil. Chemical communication in the rhizosphere plays an integral role in rhizosphere ecology by selectively influencing the growth of specific bacteria and fungi by serving as selective growth substrates and

signaling molecules, thus encouraging inter and intra-species competition, resulting in a highly sensitive environment. Therefore explaining why genotypic differences did not influence decomposition like it did in the rhizosphere.

In Chapter 4, the decomposition of AG and BG tissues of ZT and TAN genotypes was assessed. Though differences in polyphenol content between the tissues of TAN and ZT genotypes did not significantly affect respiration rates or cumulative C loss, differences between AG and BG tissues did occur. Averaged across genotype, respiration rates and cumulative C loss for AG soils were significantly higher than for BG soils. Additionally, the microbial community structure was significantly different between AG and BG soils. Notably, Gram- bacteria and fungi were dominant in AG soils, while Gram+ and actinobacteria were associated mainly with BG soils. Further, the microbial growth and enzyme activity patterns of AG soils, over time, were indicative of copiotrophic microorganisms exhibiting inefficient R-growth strategies. Moreover, soil microbial communities in BG soils produced proportionately more cell biomass per unit of C degraded than microorganisms in AG soils, indicating that biochemically complex C compounds have the potential to be degraded with a higher efficiency than what has been previously thought. In addition, differences in polyphenol content between the tissues of TAN and ZT genotypes did not significantly affect respiration rates or cumulative C loss and may be an indication that C:N ratios are more important in regulating decomposition dynamics than the chemical structure of C compounds.

### 5.2. Future Research

Results of Chapter 3 suggested a possibility of increased SOM priming in the rhizosphere of ZT lentil genotypes. In order to determine microbial utilization of SOM under ZT genotypes, the simultaneous monitoring of the release of C and N is necessary. Labelling different plant and soil fractions with $^{13}C$, $^{14}C$ and $^{15}N$ will enable us to clearly identify the source of released C and N. The dynamics of mobilization and immobilization of nutrients and its evaluation with microbial activities not only elucidate priming effects, but enable us to better understand the mechanisms involved in soil C stability.

Results of Chapter 4 suggest that BG residues are processed more efficiently than AG residues thus resulting in the potential for increased soil C retention through microbial necromass.

As this study is in contradiction to current literature on soil C stability through substrate quality and microbial activity (Cotrufo et al., 2013, 2015; Kallenbach et al., 2015, 2016), it warrants further investigation. With the use of isotopically enriched plant litter, the allocation and distribution of decomposition products can be better understood. Further, determination of CUE must involve a more direct method, such as the use of $^{13}C$ or $^{18}O$ analysis to better estimate microbial growth or metabolic flux analysis to measure patterns of $^{13}C$ position-specific $CO_2$ production (Geyer et al., 2018).

# 6. LITERATURE CITED


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