

CRANFIELD UNIVERSITY

AGNESE MANCINI

COVER CROPS FOR SUSTAINABLE MAIZE PRODUCTION

SCHOOL OF WATER, ENERGY AND ENVIRONMENT

Full time PhD
Academic Year: 2014 - 2017

Supervisors: Dr Mark Pawlett and Dr Lynda K. Deeks
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This thesis is submitted in partial fulfilment of the requirement for the
degree of Doctor of Philosophy

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ABSTRACT

Conventional maize-after-maize in the UK is frequently associated with soil compaction, runoff and soil erosion, due to a late autumn harvest and a post-harvest management that leaves minimal soil protection. Consequently, maize cultivation leads to on-site soil degradation and off-site environmental pollution. Soil conservation practices such as cover cropping could improve the sustainability of maize.

Two field experiments were established within commercially grown maize (Herefordshire) in two consecutive years to investigate the role of different species of cover crops in reducing runoff, preventing soil erosion and enhancing soil microbial community. Experiment 1 compared common non-cover crop post-harvest managements with *Lolium multiflorum* and *Secale cereale*. Experiment 2 compared *Lolium multiflorum* and the mixtures *Lolium multiflorum* & *Vicia villosa* and *Lolium multiflorum* & *Trifolium alexandrinum* with the traditional method of post-harvest wintered maize stubbles without cover crops.

Runoff and soil loss were monitored using enclosed erosion plots periodically sampled. Soil was tested for chemical parameters such as organic matter, organic carbon, total nitrogen, available phosphorus, and biological parameters such as microbial and fungal biomass, phospholipid fatty acid analysis and multiple substrates induced respiration. Additionally, during Experiment 2 easily available glomalin related soil proteins and aggregate stability were measured.

Results showed a trend in the reduction of runoff and soil erosion. However, no significant differences were recorded. Overall, the impact of cover crops on soil microbiology was limited. Cover crops had a priming effect during Experiment 1 that led to a reduction of organic matter and organic carbon due to microbial activity. In Experiment 2 the relative abundance of fungi increased in the cover crop treatments. This was possibly due to the decrease of easily available nutrients and the increase of plant root debris, which are more available to fungi rather than bacteria.

Cover crops seemed a practical option to improve the sustainability of maize production due to lack of an adverse impact on maize yield and the encouraging results on runoff, soil erosion and soil fungi. However, the effects were limited and further investigation is needed to prove the impact of cover crops in the long-term.

Keywords:

Soil microbiology, PLFA, aggregate stability, ryegrass, clover and vetch

ACKNOWLEDGEMENTS

I would like to thank the Wye and Usk Foundation and the Douglas Bomford Trust for sponsoring this PhD, especially Kate Speke-Adams for her interest in the project and Dr Paul Miller for his intriguing questions and comments. I am most grateful to my supervisors Dr Mark Pawlett for his expert supervision, proofreading skills and most welcomed sense of humour and to Dr Lynda K. Deeks for her infinite patience, practical skills and her professional advice that kept me focused on my project and limited my flights of fancy. A special thank you goes to Dr Robert Simmons for his guidance and support during these years and Ceri Dawson for her attention to details that taught me to be more organised

I am grateful to the maize growers Christopher and Tony Norman for allowing me to use their field for my project for two consecutive years, to Francis Dunne for his expert advice on crop agronomy and Derick for always being available when I needed help around the farm.

I would like to thank all my colleagues and friends at Cranfield University Alexandra, Iain, Sabrina, Tom, Jana, Ezekiel, Kelechi, Léa, Mudassir, Simone, Aurèle; you have made my life at Cranfield more interesting. Special thanks go to Dr Joanna Niziolowski, Dr Daniel Simms, Dr Monica Rivas Casado and Dr Ian Truckell for the stimulating discussions and expert advice. I want to thank my colleagues Alexandra Cooke and Iain Dummett for being such kind and supporting friends during the past three years.

A special thank you goes to all that helped me in the setup of the field experiments: Alexandra Cooke, Iain Dummett, Leah Sidi, Dr Oliver Pritchard, Dr Joanna Niziolowski, Dr Lynda K. Deeks, Giulia Franceschini and Camilla Franceschini; you know I could not have finished without you.

I would like to express my sincere gratitude to the laboratory technicians; thank you Maria Biskupska, Jan Bingham, Monika Jodkowska, Paul Barton, Caitlin Hedinburgh-Bailey, Tamazon Cowley for your help and guidance; it has been a pleasure working alongside you.

I would like to thank my friends Alessandra, Camilla, Carlotta, Giulia and Federica for encouraging, listening and supporting me when I needed the most; you are the most precious friends. A special thank you goes to Zygimantas for his loving care and for always believing in me. Last but not the least, I would like to thank my family: my parents Sara and Luigi, my brothers Damiano and Pietro, my sister Chiara and all my extended family for making me feel the most welcome every time I am back home.

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LIST OF ABBREVIATIONS

AD	Anaerobic digestion
AM	Arbuscular mycorrhizae
AMF	Arbuscular mycorrhizal fungi
AP	Available phosphorus
BSA	Bovine serum albumin
C	Carbon
CFU/s	Colony-forming unit/s
EC	Electrical conductivity
F/B	Ratio between fungi and bacteria
FAMES	Fatty acid methyl esters
GC	Gas chromatography
GHGs	Greenhouse gases
GPRS	General Packet Radio Service
GSM	Global Systems for Mobile Communications
LOI	Loss on ignition
MB	Microbial biomass
MBC	Microbial biomass carbon
MSIR	Multiple substrates induced respiration
N	Nitrogen
OC	Organic carbon
OM	Organic matter
P	Phosphorus
PLFA	Phospholipids fatty acid
qCO₂	Metabolic quotient
SAC	Special Areas of Conservation

SH	Soil health
SOM	Soil organic matter
SPE	Solid phase extraction
TN	Total nitrogen
TOC	Total organic carbon

1 INTRODUCTION

Intensive agriculture had and is still having detrimental impacts on the environment, reducing the ability to provide ecosystem goods and services and threatening the stability of social systems (Montgomery 2007, Pretty and Bharucha 2014). The negative aspects are due to the high through-flow of nutrients, pesticides, carbon (C) and water, the low water storage capacity, and reduced nutrient recycling capacity associated with intensive agro-ecosystems (Hendrix et al., 1992). The use of heavy machinery, conventional tillage operations and removal of crop residues increase the risk of soil erosion, compaction, capping, and organic matter (OM) depletion. Such degradation of soil health (SH) threatens agricultural production and increase its dependency on external inputs (e.g. fertilizers), creating a vicious cycle (Tilman, 2002).

It is necessary to increase agricultural productivity to sustain a growing human population while minimising soil degradation and preserving ecosystem services (Kibblewhite, Ritz and Swift, 2008). It is also essential to develop sustainable agricultural systems able to “sustain the people and maintain the land” (Doran and Zeiss, 2000). This approach is called “sustainable intensification”. Sustainable intensive systems need to enhance nutrient cycling, biological nitrogen fixation, allelopathy, predation and parasitism, to be self-sustained, rely less on external inputs and have improved nutrient cycling (Bardgett and McAlister, 1999; Pretty and Bharucha, 2014). Sustainable intensive systems need to reduce nutrient and water losses by increasing fertilizer and irrigation efficiency with precision farming and modern technologies. Crop varieties need to be selected to provide the best yield with minimum input. Soil health needs to be maintained, by eliminating accelerated soil erosion with the application of cover crops during fallow periods and/or reducing intensive tillage (Thomson et al., 2012).

Land cultivated with maize in the United Kingdom (UK) has increased by 20% in 5 years (2012-2016), driven by the increased demand of energy crops for anaerobic digestion (AD) (Defra, 2017). Maize production is exceptionally damaging to the environment due to the limited return of plant debris to the soil

and the severe runoff and soil erosion, which are caused by the limited soil cover post-harvest. Cover crops are a soil conservation practice with the potential to reduce runoff and soil erosion, increase soil C, as well as contribute to the overall health of the soil through interactions with the microbial community. Therefore, they could contribute to the creation of a maize-after-maize sustainable intensive system.

Traditionally, post-harvest techniques involved leaving only maize stubble over winter, which does not provide adequate soil protection from rainfall. Often, in these traditional systems, strips of soil are tilled cross the slope to increase infiltration and to reduce runoff (Defra, 2005). Alternatively, cover crops can be grown to improve water infiltration and aggregate stability (De Baets et al., 2011a; Perin et al., 2002; Pratt et al., 2014), reduce runoff velocity and protect the soil from the direct impact of raindrops (Burney and Edwards, 2005; Kaspar and Singer, 2011a; Liedgens, Frossard and Richner, 2004a).

This research focused on investigating whether there is any benefit from the use of cover crops for maize production on soil microbiological properties and erosion reduction. Cover crops are a very diverse group of plants. Therefore, for this study, it was necessary to individuate species that were adapted to UK climate and soils and multifunctional, i.e. able to reduce soil erosion and enhance soil microbial community. No previous studies have looked specifically at the effects of living cover crops on both soil erosion and soil microbial community in UK field trials without the addition of other soil conservation practices.

1.1 Maize use in the UK

Maize (*Zea mays*) is the grain which is mostly grown worldwide, 990.64 million tons yr⁻¹ (USDA, 2015). It has been cultivated for centuries for animal feed and human consumption. In modern times, maize is also used for biogas production through the process of AD. This practice is common in Europe (e.g. Germany) and has been encouraged in the UK through subsidies such as Feed-in tariffs and Renewable Heat Incentive (RHI). Defra sees AD plants as a critical factor

in helping the UK to achieve: i) 26% reduction of CO₂ emissions compared to 1990 levels by 2020 (Anon, 2008), ii) 15% energy production from renewable sources by 2020 (EU target), and iii) 35% reduction in organic waste going to landfill (1995 compared to 2020 levels; EU Landfill Directive; Defra, 2009).

Total area cultivated with maize in the UK has enlarged from 158,000 ha in 2012 to 194,000 in 2016 (Defra, 2016). In England, maize has grown from 171,000 ha in 2014 to 182,000 in 2016 (Defra, 2017). The increase in maize cultivation responds to an increased demand of fuel for anaerobic digestion, which has grown from 29,000 ha in 2014 to 55,100 ha in 2016 (Defra, 2017). In 2013, there are around 500 agricultural AD plants in the UK, while the National Farmers' Union aspires to have 1000 AD plants on farms by 2020 (NFU, 2013). Therefore, the advance of AD plants can be considered to be driving the increasing demand for maize production in the UK.

Biogas production via AD on a farm scale has environmental benefits and can also have economic benefits. For example, the use of cow and pig slurries to produce biogas can reduce emissions of odours, ammonia and greenhouse gases (GHGs) and produce energy (Bywater, 2011). However, the use of manures alone, due to low dry matter content, will not provide a valuable biogas yield. Co-digestion of organic waste and energy crops (e.g. maize, miscanthus, and sunflower) can boost biogas production (Bywater, 2011). Consequently, energy crops are experiencing a global increase in production (Amon et al., 2007). Maize is the most cultivated biogas crop because it gives high methane yield, 5480 m³ha⁻¹yr⁻¹ (Heiermann et al., 2009). However, high methane production can also be obtained using root crops and green forage crops like forage beet (*Beta vulgaris*; 5800 m³ha⁻¹yr⁻¹), ryegrass (*Lolium* sp.; 4060 m³ha⁻¹yr⁻¹), and wheat (*Triticum aestivum*; 2960 m³ha⁻¹yr⁻¹) (Demirel, Onay and Yenigün, 2010).

1.2 Maize cultivation and soil degradation

On-site soil degradation and off-site environmental pollution are often associated with conventional maize-after-maize systems in the UK (Boardman

2013, Tilman 2002). Frequent surface runoff and accelerated soil erosion are caused by a late autumn harvest often in wet soil conditions, which can lead to soil compaction, and by the lack of soil cover post-harvest (Section 1.3) (Posthumus et al., 2011). Erosion can occur also during maize growth, since maize plants are traditionally sown in rows 75 cm wide and they take months to reach 100% soil cover, meanwhile the soil is exposed to rainfall events. Studies have reported that narrower rows (45 cm) can result in lower erosion rates (Brant et al., 2017).

Additionally, maize rows can run parallel to the slope direction, concentrating runoff, increasing runoff velocity and erosivity. Leaves play an important role in the generation of runoff under crop canopy; leaf drips concentrate rainfall increasing its intensity exponentially increasing splash erosion and leading to runoff (Morgan 2005). Morgan, Morgan and Finney, (1984) reported an increase in soil erosion under maize (2 m height) from two to 14 times compare to bare ground due to leaf drips under simulated rainfall (50mm h⁻¹ and 100 mmh⁻¹).

In the UK, maize is mostly cultivated conventionally. Consequently, the soil is intensively tilled and large quantities of agrochemicals or organic amendments are used to increase soil fertility and to control weeds. Tillage is the physical disturbance of the soil by agricultural machinery aimed to favour plant growth (Klute, 1982). It reduces soil organic matter (SOM) through increasing the surface area of aggregates and consequently exposing stable SOM to oxidation and microbial decomposition (Aziz, Mahmood and Islam, 2013). Aggregate stability is reduced, and thereby the soil becomes more prone to erosion. Consequently, the mechanical action of tillage precludes the development of hyphal networks, decreasing fungal biomass. Tillage also reduces nematodes and earthworms populations (Baker et al., 2006; Breure, 2004), preventing the development of a mature and diverse community of soil organisms and reverting the soil to an earlier successional stage (Breure, 2004). For these reasons, microbial communities in agricultural soils are dominated by bacteria (Morriën, 2016) (Section 1.4.3.1).

Another disturbance of the soil system is due to the increasing weight of farm machinery, which causes soil compaction. Consequently, soil structure is degraded, surface runoff increases and soil erosion accelerates (Gil and Gil, 2011). Other impacts of agricultural management come from the use of fertilizers. Fertilizers (mineral and organic) are at risk of leaching. Particularly nitrates and phosphates are lost over autumn and winter, due to the extensive runoff associated with maize cultivations, and they can contaminate ground waters and shallow waters (Komainda et al., 2016). High nutrient inputs decrease soil microbial diversity (Verzeaux et al., 2016a) and are disadvantageous for symbiotic interactions such as arbuscular mycorrhizae (Verbruggen et al., 2015). On one hand, the increased availability of phosphorus (P) and nitrogen (N) in soil increases fungal C demand, which can lead to parasitism (Section 1.4.4.1). On the other hand, parasitic species can overcome mutualistic species, since parasites are better competitors for plant C in a system with surplus N and P (Johnson, 2010). Additionally, the cultivation of maize as monoculture and the repetitive use of pesticides contribute to biological imbalances and can cause shifts in the soil community by eliminating specific organisms. Such intensive systems enhance the accumulation of specific pathogens in the root zone that are better adapted to the soil habitat of maize, and that often need to be controlled by agrochemicals that further disrupt the soil food web (Breure, 2004; Kalia and Gosal, 2011; Morriën, 2016).

There is growing concern about the production of maize-after maize systems to sustain biogas generation, especially in a climate change scenario with extensive winter precipitations, which will increase the risk of runoff and soil erosion (de Vente et al. 2008; González-Hidalgo et al. 2007). This concern has been recognised by the UK government. In 2017, it reduced subsidies on maize Feed-in and RHI tariffs of Ca. 50% compared to 2014 (Ofgem, 2017a, 2017b). Additional political efforts have also been made to protect the soil. The Good Agricultural and Environmental Conditions of the new Common Agricultural Policy scheme for England comprise i) maintenance of a minimum soil cover, ii) implementation of erosion control measures, and iii) conservation of soil organic matter (Defra, 2014). This new policy will require maize growers to adopt new

practices of soil protection, such as cover cropping (Jokela et al., 2009) (Section 1.5).

1.3 The erosion process

In the natural environment, soil formation and soil loss via soil erosion nearly compensate. However, anthropogenic activities, such as agriculture and deforestation, can accelerate soil erosion processes thus resulting in a net soil loss (Montgomery, 2007a; Weggel and Rustom, 1992). Additionally, soil formation does not compensate for soil erosion, since paedogenesis varies from 0.02 to 1.9 Mg ha⁻¹ yr⁻¹ (Alexander, 1988), which is three to 40 times less than the rate of soil erosion of traditional arable land in Europe (Verheijen et al., 2009). Consequently, soils are a non-renewable resource in the human time scale (Jenny, 1980) and soil erosion is a major environmental and cultural threat worldwide (Mullan, 2013).

Soil erosion is the detachment, entrainment, transport and deposition of soil particles by physical forces such as rainfall, wind and surface water runoff (Morgan, 2005). It is a natural geological process, according to which materials eroded from highlands are deposited on lowlands, forming and renewing floodplains and plateaus, without which ancient civilisations may not have evolved (Lal, 2003). However, it is recognised that accelerated soil loss has led to land degradation and contributed to the demise of ancient civilisations, like Mesopotamia and Maya (Montgomery 2007).

1.3.1 Agents of soil erosion

Rainwater is the primary cause of erosion in Europe. However, wind erosion is a local hazard in north-west Europe (e.g. Netherlands, Denmark, southern Sweden and south-eastern England) (Riksen, Brouwer and De Graaff, 2003). Soil erosion by water is complex and multifaceted process and it occurs at multiple scales. Runoff and rain are the primary agents of detachment and transportation (Ran et al., 2012; Romkens, Helming and Prasad, 2001).

Runoff, or surface flow, erodes soil when the shear stresses (drag force) of the flow surpass soil shear strength of the soil (Bradford and Huang, 1994; Verheijen et al., 2009b). The amount of soil displaced and the size of the particles depends on volume and velocity of the runoff. Runoff volume depends on the duration of the rainfall and on the percentage of water that infiltrates into the soil; the latter depends on soil properties like hydraulic conductivity, texture, structure, presence of compacted layers and water storage capacity (Haghnazari, Shahgholi and Feizi, 2015). Depth and velocity of the runoff are affected by topography and slope characteristics (Ran et al., 2012).

Rain erodes the soil due to the displacement of soil particles by rain-splash when the shear stresses of the drops exceed the shear strength of the soil (Morgan, 2005). The quantity of soil removed depends on raindrop size and impact angle, which is determined by the direction and velocity of wind, by soil surface roughness and by vegetation cover (Helming and Prasad, 2001; Levia and Frost, 2003; Nanko, Hotta and Suzuki, 2006). The impact of rainfall also depends on the presence of water ponding on the surface and on soil erodibility. A thick layer of water protects the surface from splashing, while a thin layer has the opposite effect (Auerswald 1993).

1.3.2 On-site and off-site consequences

Soil erosion and runoff in agriculture represent a threat to the environment and agricultural productivity (Bakker, Govers and Rounsevell, 2004) since they can impair the ability of soils to deliver ecosystem services (Costanza et al., 1997; Fullen & Brandsma 1995; Verheijen et al. 2009). The topsoil, which contains the highest proportion of SOM and nutrients and is the most vulnerable to erosion. On-site, the breakdown and rearrangement of soil micro and macro-aggregates leads to degradation of soil structure, capping, and loss of OM, plant nutrients and soil C. Consequently, the soil loses its fertility, cultivable depth and water holding capacity, and subsequently yield is reduced (Arriaga & Lessy, 2003; Morgan, 2005). The off-site effects are many. Sediments accumulate in rivers and ditches, which increase flood risk, blocks canals, impacting navigation and reducing water storage capacity in reservoirs.

Suspended particles cause water turbidity, which can lead to an increase in water temperature and a decrease of oxygen dissolution and light penetration in water. The sediments impact riverine habitats such as fish spawn areas and reduce biodiversity and (Haregeweyn et al., 2012). Additionally, sediments coming from agricultural land can damage infrastructures and houses (Mullan, 2013). Erosion causes water pollution, such that the concentration of agrochemicals of the sediments can exceed that of the original soil (enrichment process) since sediments have higher sorption capacity than the original soil (Nearing et al., 2017). Consequently, C, pesticides, P and N concentrate in the water bodies causing contamination and eutrophication (Morgan, 2005). Phosphorus especially has been recognised as a principal agent of water eutrophication (Khan et al., 2014).

1.3.3 Effect of vegetation on erosion processes

The presence, structure and type of vegetation cover are closely related to the erosion process. Vegetation decreases soil erosion principally because it can intercept raindrops, reduce runoff flow velocity and increase soil aggregate stability. A multi-stratified canopy, composed by herbaceous plants, shrubs and trees, which is characteristic of a natural vegetation composition, is more efficient than a single height of plants (Casermeiro et al., 2004). The increased efficiency is due to the better interception of raindrops and leaves fall-off of the more complex canopy. Grasses and other vegetation that grow near the soil surface are the most critical strata for erosion protection (Zhongming et al., 2010).

Plant roots are another important component of soil loss reduction because they can decrease soil erodibility (De Baets et al., 2011a) (Sections 1.5.3 and 1.5.4). Plant roots together with fungal hyphae and bacterial exudates act as bonding agents that hold soil macro-aggregates together, reducing erodibility (Tang et al., 2011). In particular, fungal activity is related to the formation of soil macro-aggregates through the production of polysaccharides. The physical bounding of soil particles by fungal hyphae, and the production of hydrophobic compounds such as glomalin decrease aggregate permeability, increasing their

stability and therefore erosion (Le Guillou et al., 2011; An et al., 2013) (Section 1.4.5.1).

1.3.4 Soil characteristics

Different soil properties affect erosion. Slope topography and morphology affect the distribution of soil at different scales; soil on ridges will be eroded first and sediments deposited in concave areas (De Alba et al., 2006). At a local scale, slope inclination is directly proportionated to soil loss and the length of the slope determines where the sediments are deposited (Zingg, 1940). Soil physical, biological and chemical properties determine the amount of water infiltrating into the soil and the breakdown, transport and deposition of soil aggregates (Abu-Hamdeh, Abo-Qudais and Othman, 2006; Wischmeier et al., 1978). Soil texture is critical to soil erodibility. Soils that are highly erodible include those with a high content of silt, fine sands and expandable clays, while the size of the medium and coarse sands and the cohesion of non-expandable clay particles work to prevent soil erosion (Morgan, 2005). Soil compaction decreases the rate of water infiltration and can limit the water storage capacity of a soil profile, increasing the risk of runoff and erosion (Alliaume et al., 2014). The arrangement of soil particles and voids is fundamental to soil functioning; it can be deteriorated by soil management activities like soil tillage, which breaks down soil particles, decreases soil stability and increases soil susceptibility to erosion (Bronick and Lal, 2005b) (Section 1.3.5). Additionally, the amount of OM conditions water retention and aggregate stability, a soil with high OM content will be less susceptible to erosion (Rhoton, Shipitalo and Lindbo, 2002).

1.3.5 Soil management

Soil management affects the erosion process because it determines tillage practices and percentage of canopy cover throughout the year (Reynolds et al., 2007; Romkens, Helming and Prasad, 2001; Wischmeier and Smith, 1978). There are different forms of tillage; primary tillage is the first major mechanical manipulation of the soil which aims to turnover, breakdown and pulverise of the soil clods using a plough such as a mouldboard plough, disc plough; while,

secondary tillage refers to the preparation of the seedbed. Tillage can also be used to remove a compacted layer utilising a subsoiler, chisel plough, tine or disc implements (Morris et al., 2010). Even first time tillage has been found to cause significant erosion of stable OM (Beniston et al., 2014). However, some forms of tillage are used to increase water infiltration, For example, Defra, (2005) suggested to “rough” plough the soil (e.g. with chisel plough) after maize harvesting to limit runoff and soil erosion.

Primary and secondary tillage increase soil erosion because they break down soil aggregates, enhancing runoff transportation of particle and reduce SOM; they compact the soil, decreasing water infiltration and increasing runoff (Morris et al., 2010). They also cause a progressive displacement of the soil particles from upslope to downslope, in a process called tillage erosion (Van Muysen and Govers, 2002; Tiessen et al., 2007). It has been estimated that tillage erosion displaces the same order of magnitude of soil as water erosion (Van Oost et al., 2006). The direction, depth, and speed of tillage directly affect tillage erosion rates (Van Muysen, Govers and Van Oost, 2002). Maximum soil erosion can occur not only by ploughing in the direction of the slope but also by contouring because of the movement of soil lateral to tillage direction (De Alba et al., 2006b). De Alba et al., (2006b) found that a mouldboard plough leads to maximum erosion when tilling at 70° and not downslope (90°).

The type of plough used is crucial in determine the intensity of the erosion process; for example shallow ploughing produces less tillage erosion than ploughing with a mouldboard. Mouldboard ploughing is the most used tillage practice in conventional agriculture and it is usually referred to as conventional tillage. The plough affects the entire topsoil (up to 40 cm depths) and completely inverts the soil. It is usually followed by a secondary tillage (disks, harrows, and field cultivators) that pulverise the soil additionally to prepare the seedbed. Conventional tillage is generally associated with high water and tillage erosion rates, compacted layers and surface sealing (Govers et al., 1994). Another form of primary tillage is performed with the disc plough. The disc plough is formed by heavy discs, able to rotate. This type of plough is

mostly used in soil with a high content of clay and in soil with a significant amount of plant residues, because it does not get caught in the debris as much as the mouldboard ploughs (McKyes, 1989). Additionally, the disc only partially inverts the soil and more residues are left on the soil surface (Elliot, 1994).

A decrease of erosion and runoff is associated with the adoption of conservation tillage practices (non-inversion tillage) such as minimum tillage (reduced tillage) and zero-tillage (no-till) (Alliaume et al., 2014; Auerswald, Mutchler and McGregor, 1994; Bradford and Huang, 1994). Minimum tillage is a form of tillage where the soil is disrupted less than conventionally, for examples by using a chisel plough instead of a mouldboard plough, which is formed by long shanks, which loosen the soil without inverting it (McKyes, 1989) or by performing a shallow primary tillage with a disc harrow. Another critical aspect of minimum tillage is the increased crop residues left on the surface, which can be achieved by adopting strip tillage. According to this tillage technique, the field is tilled in strips, part of it (more than 2/3) is left undisturbed, and 60–75% of crop residues is left on the surface (Morris et al., 2010).

No-till is the practice where the soil remains undisturbed and crop is planted directly in the residues of the previous crop by direct drilling (Morris et al., 2010), this practice is associated with reduced soil erosion. Lal, (1987) reported that soil erosion in conventional tillage maize (mouldboard ploughing followed by disc harrowing before planting) was 42 times larger than in no-till maize. Derpsch et al., (2010) reported that in South-America medium-large farms after 10 years of no-till adoption reported a reduction of soil erosion, an increase of OM, crop yield and decrease in labour. Basic et al., (2004) reported a 40% decrease of soil erosion in no-till maize compare to conventional tillage (up and down the slope). Prasuhn, (2011) reported that soil loss from no-till field ($0.12 \text{ t ha}^{-1} \text{ yr}^{-1}$) was 90% less compare to mouldboard plough fields ($1.24 \text{ t ha}^{-1} \text{ yr}^{-1}$). Mhazo et al., (2016) analysed 41 worldwide studies and reported that soil losses was 60% less under no-till compared to conventional tillage. Due to the success of no-till from 1999 to 2009 there has been 60% increase in no-till farming from 45 million ha to 111 million ha worldwide (Derpsch et al., 2010).

1.4 Soil biota and ecosystem functions

Soil delivers a diverse range of ecosystems services. It provides and purifies water and regulates gaseous exchange, mainly greenhouse gases, like carbon dioxide. Soils support plant growth, recycle raw materials and nutrients, host an incredibly diverse genetic resource and are the surface on which we live a built on (Brady and Weil, 2016). All these functions are related to biological processes, which are, however, limited by the chemical and physical environment (Kibblewhite, Ritz and Swift, 2008).

Ecosystem function has been historically linked to the presence of keystone organisms, e.g. earthworms. For example, plants influence the amount of C sequestered and earthworms can rearrange soil structure and enhance plant tissue decomposition (Turmel et al., 2014). Soil microorganisms are fundamental to the function of the soil ecosystem; they are the first colonisers of parent material and can enhance the degradation of the rocks. Fungi and bacteria are responsible for the provision of fundamental ecosystem services like C sequestration, nutrient cycling, soil formation and soil structural integrity (Breure, 2004). Fungal hyphae and bacteria exudates act as a bonding agent that holds soil macro-aggregate together (Tang et al., 2011) (Section 1.4.5.1) but they are also responsible for their breakdown since SOM it is also the primary energy substrate (Section 1.4.5.2).

There is no clear indication in the literature at which level of biodiversity loss ecosystem function would be impaired. On one hand, there is a genuine functional redundancy among soil organisms, and species loss should not necessarily affect soil biological and biochemical processes (Allison and Martiny, 2008; Borer et al., 2005). On the other hand, it is likely that organism with similar function present different environmental tolerances, and they are essential to guarantee soil functionality in different abiotic conditions (Beare et al., 1995). Moreover, the majority of soil function is dependent on soil biota interaction and on soil food web, which are sensitive to species loss (de Vries et al., 2013).

1.4.1 Soil microbial community

Soil microbial community comprises of populations of different microorganisms living in the same habitat (Morris and Blackwood, 2015a). The soil is predominantly made up of mineral material with a non-uniform distribution of organic material. Thus, the majority of the microbial biomass (MB) is mostly in a dormant state due to the lack of resources (Morris and Blackwood, 2015a). However, there are hotspots of microbial activity focused on areas such as the rhizosphere, due to the input of labile organic compounds, faecal matter and decomposing plant debris, which are rich in SOM and nutrients (Kuzyakov and Blagodatskaya, 2015). In these areas, microbial activity is more significant compared to those of the bulk of soil and microbiology requires constant input of nutrients, which can result in decomposition of OM (rhizosphere priming effect) (Kuzyakov and Blagodatskaya, 2015).

The soil microbial community evolves over time throughout a natural succession of populations. Colonisers of habitat patches are typically opportunistic species, effective in the use of resources, which are eventually replaced by species more competitive in low nutrient environments (Balestrini et al., 2015). Consequently, the soil habitat consists of a mosaic of patches in continuous evolution (Wu and Loucks 1995). The composition of root exudates and litter influences the structure of the microbial community (Haichar et al., 2014; Kaiser, Kleber and Asefaw, 2015). Additionally, soil community structure is influenced by ecological, historical, geographical and evolutionary contingencies (Belyea and Lancaster, 1999). Some of ecological aspects that are fundamental in shaping a community are:

- Sprengel–Liebig Law of the Minimum, which state that the resource that is limited, will limit the growth of organisms (von Liebig 1840 in Browne, 1942).
- Shelford's Law of Tolerance, whereby a population growth is limited by the range of environmental factors (temperature, pH, water presence, oxygen levels), above and below which the species cannot survive (Shelford 1911 in Shelford, 1931).

- *K* versus *r*-selection continuum. An *r*-species reproduces exponentially if a resource is available, it is very effective into exploiting the resource, and its population declines rapidly after that. A *K*-species has more stable number of individuals and is adapted to survive where resources are limited (MacArthur and Wilson 1967 in Pianka, 1972).
- Exploitation mechanism: interspecific and intraspecific competitions, predation, herbivory, parasitism and pathogenesis (Morris and Blackwood, 2015a). Intraspecific competition especially has a stronger effect on population regulation since the organisms of the same species compete exactly for the same resources (Morris and Blackwood, 2015a).
- Positive interactions between soil microorganisms, such as mutualism, affect the composition of the community. The most common mutualistic relationship is the mycorrhizae, which are an association between fungi and plant roots (Section 1.4.4.1).
- Extinction, mutation and species dispersal (Hanson et al. 2012). Physical barriers which prevent species dispersal can influence the composition of the community (Leibold et al., 2004).

1.4.2 Fungi

A very diverse range of organism composes the Kingdom Fungi, Eumycota. They represent the majority of eukaryotic microorganisms in term of biomass (Balestrini et al., 2015). Fungi species richness is estimated to be around 5-6 million species (Taylor et al., 2014). Fungi can be divided into three functional groups: i) yeasts, which are mono-cellular, ii) moulds, which are filamentous organisms involved into SOM degradation, and iii) mushroom fungi, which are filamentous fungi that can form an above-ground fruiting body and symbiotic relationships with plants (Brady and Weil, 2016). Moulds and mushroom fungi are formed by hyphae, extended filamentous structures.

Fungi species composition and distribution vary according to soil pH, temperature, moisture content and nutrient availability; biomass is related primarily to nutrient availability (N and P; Section 1.4.3.1) (Fierer et al., 2009). Plant community composition also influences the structure of the fungal

community since most fungi feed on plant material (Reynolds et al. 2003). Their distribution is also limited by their ability to disperse. Passive fungal dispersal is due to the spores, while the growth of hyphae can be considered an active fungal dispersal; in this case, the hyphae are often thicker than usual (Morris and Blackwood, 2015a). At the microscale, fungi are found primarily on the surface of aggregates and in macropores (Frey, 2015). Fungi are involved in i) decomposition and mineralisation processes (Section 1.4.5.2), ii) control of soil structure (Section 1.4.5.1), iii) pathogenic and symbiotic interactions with plant roots (Sections 1.4.4), and iv) regulation of above-ground biodiversity (Balestrini et al., 2015). Fungi drive the decomposition process, assisted by other microorganisms such as Protista, e.g. Amoebozoa (Adl and Gupta, 2006). Also, fungi support C sequestration and nitrogen fixation throughout lichen symbiosis with algae and cyanobacteria (Taylor and Sinsabaugh 2015). Some fungi can obtain N feeding on nematodes (Barron, 2003).

1.4.3 Prokaryotes

Soil prokaryotes comprise bacteria and archaea. Their number and species diversity are extensive (Whitman, Coleman and Wiebe, 1998). Estimates suggested that there are 10,000 different species of microorganisms per cm³ of soil (Breure 2004, Fierer & Lennon 2011). This variety is due to their size (1-10 µm), fast reproduction and adaptation to the soil habitats. It has been calculated that in a gram of soil the number of prokaryotes varies from billions to trillions (Sikorski, 2015). Prokaryotes are both autotrophic (e.g. cyanobacteria) and heterotrophic; while most bacteria are heterotrophic. Archaea represent 10% of the soil MB, and in contrary to the majority of soil organisms, bacteria included, their abundance tends to increase with depth (Fierer, Schimel and Holden, 2003). Soil prokaryotes are fundamental to the function of the ecosystem. They are involved in SOM transformation nutrient cycling and nitrogen fixation, soil remediation and disease suppression (Lal, 2016).

The majority of bacteria are found in the topsoil (0-30 cm), with a decrease in biomass and species diversity around 10-25 cm (Frey, 2015). At the aggregate scale, 80% of prokaryotes are found in pores with a diameter of 2.5 µm to 9 µm

(Frey, 2015). Species diversity is highly correlated to changes in soil pH (Chu et al., 2010). Bacterial passive dispersal is due to the movement of water in the soil matrix (Morris & Blackwood, 2015). Bacterial active dispersal can be induced by the presence of roots, seeds, fungal spores and SOM (Compant, Clément and Sessitsch, 2010). During the dormant stage, the bacterial cells reduce their size and their biochemical activity to survive until favourable conditions return. Inactive cells are a form of dispersal as well and a way to colonise new habitats (Morris & Blackwood, 2015). Even if there is great phylogenetic and physiological diversity within bacteria, some abundant bacterial phyla can be broadly divided into copiotrophic or *r*-strategists and oligotrophic or *K*-strategists (Meyer, 1994). Copiotrophic bacteria are favoured when there is an abundance of labile C that they can easily exploit and use to maximise their growth. Oligotrophic bacteria are better competitors when C resources are limited or not readily available (Fierer, Bradford and Jackson, 2007). Fierer et al., (2007) found that C mineralisation rate was a strong predictor (30%) of Acidobacteria, β -Proteobacteria and Bacteroidetes. Acidobacteria decreased with the increase of C mineralisation rates, showing oligotrophic attributes; β -Proteobacteria and Bacteroidetes increased with the increase of C mineralisation rates, showing copiotrophic traits. Most bacteria can also be divided by the Gram-staining procedure into Gram-positive and Gram-negative bacteria (Gram, 1884). Gram-positive bacteria have a cell wall made of peptidoglycan (40-80%), teichoic and teichuronic acids, while Gram-negative bacteria have an additional outer membrane made of phospholipids, lipoproteins, lipopolysaccharides, and proteins (Jiang et al., 2004). Gram-positive tend to be *K*-strategists, while Gram-negative *r*-strategists (Finney, Buyer and Kaye, 2017).

1.4.3.1 Fungi and bacteria dominance

The ratio between fungi and bacteria (F/B) can differentiate microbial communities. The dominance of fungi or bacteria is not easily explained by a single soil property but mostly by a combination of factors. In conventional agricultural systems, bacteria dominate over fungi because tillage disrupts fungal hyphae, while it does not affect bacteria as they are formed by individual

cells (Hendrix et al., 1986). Fertilizers homogenise nutrient status of the soil, which makes fungi lose the advantage of being able to reach hotspots of soil fertility. Additionally, bacteria are more effective in exploiting the newly exposed SOM by tillage (Holland and Coleman, 1987). On the contrary, no-till favours fungi over bacteria because hyphae are not disrupted and also because crop residues are concentrated on the soil surface (Breure, 2004).

Fungi dominate the microbial activity in soils with low pH and low nutrient content, e.g. forests, while bacteria dominate in alkaline soils (Joergensen and Wichern, 2008). The ability of fungi to be better competitors in low nutrient environments is due to their ability to explore the soil matrix in search of nutrients and to their stoichiometry, which has a higher C/N compared to bacteria. Thus, fungi will dominate environments where N is limiting (Strickland and Rousk, 2010). Nevertheless, that is not always the case, and overlapping occurs (Strickland and Rousk, 2010).

1.4.4 Maize rhizosphere

The rhizosphere is the area surrounding the roots. It ranges only for few millimetres in the soil matrix from the plant roots, nevertheless it is a hotspot for microbial activity and diversity (Jones and Hinsinger, 2008). Plants and root microbiome live in close contact to the extent that they have been considered as one “superorganisms” (Van Der Heijden, Bardgett and Van Straalen, 2008).

Plants provide 20-50% of their photosynthate to the microbes in the form of root exudates via rhizodeposition (Dennis, Miller and Hirsch, 2010), influencing the development of a specific community of organisms. It has been found that maize root microbiome has less microbial diversity compared to the rest of the soil (García-salamanca et al., 2012). Li et al. (2014) found that Betaproteobacteria, Firmicutes, Bacteroidetes and *Cyanobacteria* were most common in the maize rhizosphere compared to the bulk soil. Root microbiome stimulated by the root exudates can suppress plant diseases by competing with the bacteria responsible for the condition (Balestrini et al., 2015). For example, maize roots exude an antimicrobial benzoxazinoid that attracts rhizobacteria

Pseudomonas, which are plant promoting bacteria (Ahemad & Kibret, 2014; Berendsen et al., 2012). Certain species of the root microbiome produce compounds (hormones) that stimulate plant growth. These are called plant growth-promoting rhizobacteria, among them *Nonomuraea*, *Thiobacillus* and *Bradyrhizobium* are found to be more common in the maize rhizosphere compared to the bulk soil (Yang et al., 2017). Maize development stage has been found to affect the bacterial community, especially the biomass of Bacteroidetes, Proteobacteria and Actinobacteria (Yang et al., 2017). During the early phase of maize plant development, *r*-bacteria are dominant, while *K*-bacteria are more common in the root zone of mature plants (Cavaglieri, Orlando and Etcheverry, 2009).

Fungal communities, also establish relations with the roots of the rhizosphere, although, the nature of the interaction is not well-known (Balestrini et al., 2015). In addition to mycorrhizal symbioses, some fungi (Sebacinales) can interact with the roots without forming a mycorrhizal; this type of fungi is called endophytes (Balestrini et al., 2015). This interaction has been found to have beneficial effects on the plant, including enhancing plant growth and decreasing fungal infections (Seghers et al., 2004). Common endophytes of maize are bacteria such as *Enterobacter* spp. and the betaproteobacterial *Burkholderia* spp. (McInroy and Kloepper, 1995) and fungi such as *Trichoderma* spp., a saprotrophic fungus, which acts as a biocontrol for many fungal plant pathogens. *Trichoderma* biocontrol action is due to i) direct competition for resources, ii) production of cell-wall degrading enzymes, iii) antibiosis and iv) induction of plant defence responses (Harman et al., 2004).

1.4.4.1 Mycorrhizae

The symbiosis of fungi with the roots of higher plants is called mycorrhizae. Mycorrhizae are mutualistic associations that occur for the majority of plants and are the oldest plant-fungus relationship and the most critical symbiosis in terrestrial ecosystems (Brundrett, 2002). The association is facultative for the majority of plants (Graham and Eissenstat, 1994), while mycorrhizal fungi do not survive without a host. Mycorrhizae are also found among non-vascular

plants, e.g. bryophytes and sporophytes (Bonfante and Genre, 2008). The plant provides sugars, in the form of hexose, to the fungi which in turn produce macronutrients like P and N, water and micronutrients like Zn (Bücking and Kafle, 2015; Johnson, 2010). Mycorrhizae also protect the plant from biotic and abiotic stresses, e.g. pathogen infection and pollution (Duponnois et al., 2005). Most agricultural plants forms mycorrhizal associations (Table 2).

There are two principal types of mycorrhizae: endomycorrhizae and ectomycorrhizae (Table 1). Endomycorrhizae, the primary form of symbiosis, extend their hyphae inside the root cells of the plant. Endomycorrhizae comprise of arbuscular mycorrhizal fungi (AMF), and ericoid mycorrhizae, which are in symbiosis with the plants of the genus *Ericales*. Ericoid mycorrhizae are more common in cold and wet climates, where litter has a high C/N. Glomeromycota fungi and the majority of all land plants form an arbuscular mycorrhizal symbiosis (Schübler et al., 2001). Arbuscular mycorrhizal fungi represent on average the 5-10% of the soil MB (Balestrini et al., 2015), they can germinate without a host plant, but they cannot produce spores without the symbiosis. Arbuscular mycorrhizal fungi are vital for the C cycle; they slowly release plant-derived C to the bacteria and fungi of the (myco-)rhizosphere (Drigo et al., 2010). They also produce hydrophobic compounds that affect the stability of soil aggregates (Section 1.4.5.1) and are able to reduce the growth of agricultural weeds (Veiga et al., 2011).

Ectomycorrhizae (ECM) infection grows inside the root but not inside the cells. Amongst the ECM fungi are the mushroom fungi. This association is more common in litters with a medium C/N, while AMF are more common in dominate warm environments where litter has low C/N. It has been demonstrated that mycorrhizal fungi transfer N to the plants. However, C is directly traded for P (Fitter, 2006; Smith et al., 2011). The relationship between plants and mycorrhizal can be explained by the trade balance model (Strickland and Rousk 2010). The model is based on P and N availability. If nitrogen is available, but P is still limited, both plant and fungus will benefit from the symbiosis. Though, if P is readily available, the plant would not benefit from the

symbiosis, while the demand for C from the fungus will increase, shifting the mutualistic symbiosis to parasitism. If N is limited, and P is in surplus, plant and fungi will compete for nutrients, but the symbiosis will not be detrimental for the plant because the growth of the fungi would be limited by the N availability (Strickland and Rousk 2010). Other types of mycorrhizae are orchid mycorrhizae and Ericoid mycorrhizae (Table 1).

Table 1 Summary of mycorrhizal symbiosis. Adapted from Heijden et al., (2015).

Symbiosis characteristic	Mycorrhizal type	Major groups of plants	Fungal identity
Endophytes	Arbuscular mycorrhiza	Most herbs, grasses and many trees, many hornworts and liverworts (74% of all plant species)	Glomeromycota (300–1600 different taxa)
	Orchid mycorrhiza	Orchids (9% of all plant species)	Basidiomycota (25000 taxa)
	Ericoid mycorrhiza	Ericaceae, some liverworts	Mainly Ascomycota, some Basidiomycota (150 taxa)
Ectophytes	Ectomycorrhiza	Pinaceae and Angiosperms (mostly shrubs and trees, mostly temperate) , some liverworts (2% of all plant species)	Basidiomycota and Ascomycota (20000 taxa)
	Non-mycorrhizal	Brassicaceae, Crassulaceae, Orobanchaceae, Proteaceae	

Table 2 Example of plant species and the type of mycorrhizal association.
Adapted from Akhmetzhanova et al., (2012).

Family	Species	Mycorrhizal type
Alliaceae	<i>Allium caspium</i>	Arbuscular mycorrhiza
Apiaceae	<i>Carum carvi</i>	
Asteraceae	<i>Acroptilon repens</i>	
	<i>Antennaria caucasica</i>	
Brassicaceae	<i>Vicia cracca</i>	
	<i>Vicia faba</i>	
	<i>Vicia multicaulis</i>	
	<i>Trifolium ambiguum</i>	
	<i>Trifolium aureum</i>	
	<i>Trifolium badium</i>	
	<i>Trifolium trichocephalum</i>	
	<i>Glycine max</i>	
	<i>Medicago falcata</i>	
	<i>Medicago lupulina</i>	
	<i>Medicago sativa</i>	
	<i>Lotus corniculatus</i>	
	<i>Lotus tenuifolius</i>	
	<i>Brassica napus</i>	
	<i>Brassica oleracea</i>	
	<i>Braya purpurascens</i>	
Linaceae	<i>Linum perenne</i>	Arbuscular mycorrhiza
	<i>Linum sibiricum</i>	
	<i>Linum trigynum</i>	
	<i>Linum usitatissimum</i>	
Pinaceae	<i>Abies alba</i>	Ectomycorrhiza
	<i>Abies balsamea</i>	
	<i>Abies grandis</i>	

Family	Species	Mycorrhizal type
Pinaceae	<i>Abies magnifica</i>	Ectomycorrhiza
	<i>Pinus monticola</i>	
	<i>Pinus mugo</i>	
	<i>Pinus nigra</i>	
	<i>Pinus occidentalis</i>	
Poaceae	<i>Lolium multiflorum</i>	Arbuscular mycorrhiza
	<i>Lolium perenne</i>	
	<i>Lolium subulatum</i>	
	<i>Avena grandis</i>	
	<i>Avena nuda</i>	
	<i>Avena strigosa</i>	
	<i>Zea mays</i>	
	<i>Triticum aestivum</i>	
	<i>Triticum dicoccon</i>	
	<i>Triticum durum</i>	
	<i>Triticum monococcum</i>	
	<i>Triticum polonicum</i>	
	<i>Triticum spelta</i>	
	<i>Triticum vulgare</i>	
	<i>Hordeum brevisubulatum</i>	
	<i>Hordeum sativum</i>	
	<i>Hordeum vulgare</i>	
	<i>Pennisetum setaceum</i>	
	<i>Pennisetum villosus</i>	
Scrophulariaceae	<i>Veronica officinalis</i>	
Solanaceae	<i>Solanum lycopersicum</i>	
	<i>Solanum nigrum</i>	
	<i>Solanum tuberosum</i>	

1.4.4.2 Pathogens

Maize microbial pathogens are many and diverse; they range from virus infections such as Rice Black-streaked Dwarf Virus (Li, Xu and Zhang, 2011) and Sugarcane Mosaic Virus (Wu et al. 2013) to fungal infections. Maize debris is the primary inoculum of fungal infection (Lukas et al., 2014). *Aspergillus flavus* is a fungus that causes ear rot and it contaminates the grains with aflatoxin B1, the most significant carcinogenic compound in nature (Castegnaro and McGregor, 1998). *Fusarium* spp. (*F. graminearum* and *F. verticillioides*) are another major fungal pathogen, dangerous for human and animal health, due to the production of mycotoxins such as fumonisin, deoxynivalenol, and zearalenone. They cause severe rotting (red and pink ear rots) and wilting that decrease maize yield (Czembor et al., 2015; Oren et al., 2003). These species are positively affected by the presence of residues on the ground surface, rainfall, and mild winter conditions. Competition with other fungi on maize residues also plays a vital role (Lukas et al., 2014).

Joergensen and Wichern (2008) reported that the type of mycorrhizae dominating in a soil affects the ratio between fungi and bacteria (F/B). Ergosterol (Grant and West, 1986) and the phospholipid fatty acid (PLFA) marker 18:2 ω 6,9 give an estimation of ectomycorrhizae and saprotrophic fungi biomass, but cannot be used to investigate arbuscular mycorrhizal fungi (Olsson et al., 2003). Therefore a change in the F/B will be detected in an ectomycorrhizae-dominated environment compared to an environment dominated by arbuscular mycorrhizal fungi (Strickland and Rousk, 2010).

1.4.5 Soil erosion prevention

1.4.5.1 Aggregate stability

Soil microorganisms affect soil erodibility through their effects on aggregate formation, stability and arrangement. Aggregate stability is the ability of a soil aggregate to maintain its integrity when exposed to disruptive forces (Vergani and Graf, 2016). Tisdall & Oades, (1982) classified soil aggregate into three classes: micro-aggregates (0.002 - 0.02 mm), meso-aggregates (0.02 - 0.250

mm) and macro-aggregates (> 0.25 mm). Micro-aggregates form by the flocculation of colloids (clay minerals, oxides of aluminium, SOM) and they are stabilised by the sticky nature of bacterial exudates and polysaccharide capsules (Schutter and Dick, 2002). Soil OM and microbial colonies are usually the core of meso-aggregates (Voroney and Heck, 2015). Macro-aggregates can form by the combination of micro-aggregates (Six et al., 2004). Macro-aggregates can also form around particulate OM. In this case, the formation of macro-aggregates precedes the formation of micro-aggregates, which are formed, lately, after the OM is decomposed into more stable forms of C by soil microbiology. Soil macro-aggregates are in continuous evolution, forming or being disrupted as the organic carbon (OC) is used by the microbial activity. Hence, aggregate stability is highly correlated to OC stability, and microbial activity (Rillig et al., 2001) and MB (Sparling, Shepherd and Kettles, 1992). Plant roots, root exudates, fungal hyphae and bacteria exudates act as bonding agent that hold soil macro-aggregates together (Tang et al., 2011). In particular, fungal activity is related to soil macro-aggregates formation and stability, because of i) the production of polysaccharides, ii) the physical bounding of soil particles by fungal hyphae, and iii) the production of hydrophobic compound that decrease aggregate permeability (Ternan et al. 1996; Le Guillou et al. 2011). An example of a hydrophobic compounds are glomalin related soil proteins, a class of molecules related to glomalin an iron-binding glycoprotein produced by AMF (Bronick and Lal, 2005b). Glomalin related soil proteins have been shown to be strongly correlated to aggregate stability and are found on the surface of soil aggregates (Brady and Weil, 2016). Aggregate stability is correlated soil erodibility since it affects soil cohesion, strength (Ekwue, 1990; Fattet et al., 2011) and soil permeability (stable aggregates maintain the soil porosity structure allowing free movement of water and air in the soil, increasing water infiltration) (Bronick and Lal, 2005a).

1.4.5.2 SOM decomposition and mineralisation processes

Soil organisms are involved in SOM dynamics, which affects soil erodibility by influencing soil water holding capacity, soil structure, and water infiltration (Lehmann and Kleber, 2015). Different theories have tried to explain the origin

and composition of SOM. According to the humification theory (Kononova, 1966), SOM is produced through the humification process which transforms plant and animal debris into dark compounds of large molecular weight. This theory is based on a series of extractions: the sodium hydroxide (NaOH) alkaline extraction, followed by the acid extraction. Materials extracted by NaOH usually have a dark colour and are called “humic acids”, which are weak aliphatic and aromatic organic acids soluble under alkaline conditions. The solution is then acidified and an orange coloured material extract, called “fulvic acid”, which are weak aliphatic and aromatic organic acids soluble under all pH conditions. The insoluble material remaining is called “humin”, which is believed to be composed of macro-organic substances. However, this theory has been criticised because there no proof of the existence of “humic acids”, “fulvic acid”, and “humin”, in the soil (Horwath, 2015) and consequently, remains contradictory (Lehmann and Kleber, 2015).

A more modern theory is the selective preservation theory (Kelleher and Simpson, 2006; Sollins, Homann and Caldwell, 1996). According to this theory, plant and animal debris are composed of a combination of substances, which are biodegradable at different rates due to their molecular composition. Proteins, particulate organic matter, lipids, and other components with a relatively high C/N, are readily degraded by the microbial community and do not form stable forms of SOM. The majority of the SOM is made of refractory materials, like polymers containing aromatic rings such as lignin and waxes, which can be decomposed by specialised fungi (e.g. Basidiomycota). However, the topsoil (0-30 cm), which contains the majority of SOM, has insufficient lignin content to support the selective preservation theory (Kögel-Knabner, 2000). Moreover, studies proved that lignin is degraded relatively rapidly in the soil (Kögel-Knabner, 2000) and that old OM (20–100 years) predominantly contains polysaccharides and proteins, which are easily degradable (Gleixner, Poirier and Bol, 2002). At most, the recalcitrance of some components can explain the delay in early decomposition. However, for the preservation of SOM in the long term, environmental conditions become more relevant (Dungait et al., 2012; Lehmann and Kleber, 2015; Schmidt et al., 2011) and the soil habitat can

provide chemical and physical protection from microbial degradation preserving easily degradable compounds..

The stability of SOM is related to the stability its major component SOC (approximately 50%), which is related to the activity of the microbial community (Pribyl 2010). Living MB is about 4% of SOC and it has been estimated that 80% of SOC could be composed by the microbial necromass (Liang & Balser, 2010). A form of SOC preservation is encapsulation inside aggregates formed by the action of microbial cells and root exudates. The formation of small aggregates can exclude microorganisms so reducing access the SOC, and limit diffusion of oxygen, water, and extracellular enzymes inside the aggregates (Doetterl et al., 2016). Soil C that bounds to mineral surfaces, like phyllosilicates, is not accessible to soil decomposers (Kleber et al., 2015). Additionally, SOC is stabilised by forming metal-organic complexes with Fe-, Mn-and Al-oxy-hydroxides (Kleber et al., 2015). Hydrophobic lipids can also form micelles, which resist dissolution in water by repelling water, increasing SOC stability. These different forms of SOC preservation can maintain SOC stable for a thousand years. Hence, SOM is “a continuum of progressively decomposing organic compounds” ranging from plant debris to extremely oxidised components, continuously degraded by the microbial community (Lehmann and Kleber, 2015). The transformation of large particles of SOC into smaller sized molecules of increased polarity and solubilisation increases the probability of SOC reacting with mineral surfaces and of being incorporated into aggregates, forming a reservoir of SOC that is fundamental to how the soil ecosystem functions (Lehmann and Kleber, 2015).

1.4.5.1 Soil health

Public and governments concern about environmental sustainability and the role which soils play into the provision of ecosystems services has pushed the scientific community to define and assess soil quality (SQ) and soil health (SH) (Carter, 2002a). Unlike for water and air, SQ and SH have not been officially defined, possibly due to the natural complexity of the soil ecosystem. Larson & Pierce, (1994) defined SQ as: “The capacity of a specific kind of soil to function

within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation". Soil health is a concept close to SQ, with the difference that SQ focuses on the maintenance of the soil functions, while SH stresses more on the functioning of the soil as an active living entity (Lal, 2016). Though, in the literature, the difference between the two concepts is not clear (Gil-Sotres et al., 2005). This confusion is probably due to the fact that some authors do not distinguish the terms and use them as synonyms (e.g. Doran, 2002; Doran & Zeiss, 2000; Lehman et al., 2015b). However, some authors insist on the importance of distinguish between the two terms (e.g. Lal, 2016).

The concept of soil health evolved in the world of ecology from the notion of ecosystem health. Consequently, an healthy ecosystem can be defined as "that with the ability to maintain desirable vital signs, to handle stress and to recover equilibrium after perturbations" (Rapport, 1992). According with this definition, soil health can be considered a subcategory of ecosystem health, and closely associated to plant health and human health (van Bruggen & Semenov 2000; Gil & Gil 2011). This is affirmed the great disease suppression of soils, the potential of sourcing human and animal antibiotics and the provision of essential micronutrients to diet, such as Fe and Zn (Lal, 2016).

The concept of SH as evolved with time and the relationship with ecosystem health has been lost in recent years. Doran & Zeiss, (2000) defined SH as: "the capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain biological productivity, maintain or enhance the quality of air and water, and promote plant, animal and human health". This definition highlights the importance of soil biota for the functioning of the soil ecosystem but leaves behind the ecological concept of soil as ecosystem able to maintain its functions with time and able to self-reorganisation after a change. Similarly, Lal, (2016) defined SH as "soil's capacity, as a biologically active entity, within natural and managed landscapes, to sustain multiple ecosystem services, including net primary productivity, food and nutritional security, biodiversity, water purification, and renewability, C sequestration, air

quality, and atmospheric chemistry and elemental cycling for human well-being and nature conservancy”.

Soil quality and SH cannot be used to assess the general state of the soil resource objectively, because they depend on the management goal, on the soil type, and climate region. Soil type and climate region set the limits of soil potential, and the management goal. Therefore, for the time being, they have proven to be a useful communication tools (Pellant et al., 2000; Wienhold, 2008) and they need to be considered as a functional concepts to evaluate the sustainability of management practices within the same soil or the same soil type (Doran, 2002; Parisi et al., 2003).

Soil health and quality cannot be measured directly but can be inferred by the changes soil attributes because soil parameters can be related to soil functions (Bhardwaj et al., 2011; Islam and Weil, 2000) or pedotransfer functions, which are soil properties expressed by a mathematical combination of other soil properties (Bouma, 1989). Traditionally, SQ is determined, primarily by physical and chemical indicators, while biological indicators have been used for SH. Authors that do not distinguish SQ and SH use a combination of physical, chemical and biological indicators (Karlen et al., 1994, 1997; Larson and Pierce, 1994). Ideally, all soil properties should be measured and multiple measurements in time should be done, due to the natural variability of soil properties. However, since this practice would be extremely costly and time-consuming (Kibblewhite, Ritz and Swift, 2008), the majority of the studies focus on few crucial soil attributes that they consider significant for SQ or SH assessment. SQ and SH can be monitored over time dynamically or can be used to compare different management practices applied at the same time (Larson and Pierce, 1994). Indicators can be descriptive or quantitative. An example of a descriptive indicator can be the presence of signs of erosion or compaction or crop deficiency. Quantitative indicators are, for instance, soil pH and SOM.

Even if microbial characteristics have not been directly related to soil functions, soil biological indicators such as MB and composition and enzymes can be

useful indicators of SH as they are susceptible to land use and management change (Cardoso et al., 2013; Finney, Buyer and Kaye, 2017; Nannipieri et al., 2017). Different biological parameters have been used in the literature to evaluate SH, such as microbial biodiversity, F/B and MB.

The use of below-ground (microbial) biodiversity, regarding the taxonomic diversity of fungi and bacteria, has been debated as indicators of SH. It is extremely time-consuming, and it is difficult to correlate it to soil management practices. Additionally, biodiversity is never a constant entity, it is related to that particular soil, and it depends on naturally occurring processes like freeze and drought (Domsch, Jagnow and Anderson, 1983; Visser and Parkinson, 1992). Furthermore, ecosystem services related to the microbial community do not depend on microbial biodiversity; a system with higher biodiversity can be less efficient than a system with less biodiversity but more specialisation. Even if a system with larger biodiversity is more resilient to environmental changes, a threshold below which soil functioning is affected would be virtually impossible to find in nature for the moment (Kibblewhite, Ritz and Swift, 2008).

Bardgett & McAlister, (1999) used F/B to assess the ability of the ecosystem to self-regulate, as fungi are usually related to more mature and stable environments. Ratio of C in the MB and SOC, the microbial quotient, can be used as indicator of SH instead of SOC since microbial biomass responds more quickly to change in soil management than soil C (Anderson & Domsch, 1989). The metabolic quotient (qCO_2), the microbial respiration per unit of microbial biomass, is sometimes used to assess stress. An increase in qCO_2 indicates that more energy needs to be used to maintain biomass instead of growing, indicating a situation of stress (Anderson & Domsch, 1993). Microbial quotient and qCO_2 can be used to create a “baseline performance” of the microbial community (Anderson 2003). Changes from the baseline could represent an improvement or degradation of SH. For instance, if qCO_2 decreases, more energy is available for MB production, therefore microbial quotient should also increase (Anderson, 2003).

Authors that consider SH as a subcategory of ecosystem health measure it using ecological characteristics. For example, microbiological responses to a disturbance or stress, diseases suppression, self-regulation, stability, resilience and integrity of the soil organism community and of the ecosystem services they provide (Aziz et al. 2013). van Bruggen & Semenov, (2000) suggested to monitor microbiology after applying a stress to measure SH, the parameters proposed were the ratio of colony-forming units (CFUs) to total microscopic counts, the ratio of copiotrophic to oligotrophic CFU and qCO_2 .

1.5 Cover crops

Cover crops are “close-growing crops such as grasses, brassicas, legumes, or cereals that are used primarily to provide seasonal protection against soil erosion and for soil improvement” (Unger and Vigil, 1998). The term cover crops is often associated with the terms catch crops, while the terms companion crops, relay crops, and intercrop refer to specific cover crop uses. Catch crops are cover crops used explicitly to uptake nutrients, which otherwise would be leached over autumn and winter. Companion crops, relay crops and intercrops are synonyms that refer to plants that are grown with the principal crop to increase its productivity by acting as a source of nutrients or pest control. Also, they can be used for animal and human feed, especially in underdeveloped countries (Mhlanga et al., 2016).

Cover crops sowing technics vary, cover crops can be under-sown in summer, at the same time or few weeks after sowing the main crop, or they can be sown post-harvest in autumn (Lesoin, 1991; Unger and Vigil, 1998). They can be composed of one species or by a mixture of plants, chosen depending on the type of soil, climate and management goal. Mixtures are generally multifunctional (Tosti et al., 2014), while pure stands are planted for a specific purpose such as erosion control, soil nutrient management, and biological pest control (Burney and Edwards, 2005). Cover crop grasses are usually broadcast, whereas legumes and cereals can be drilled.

1.5.1 Cover crops adoption

The use of cover crops worldwide for soil improvement is ancient. There is testimony that the Romans were using them to improve soil fertility and to protect the soil from erosion (Montgomery, 2007). There has been a renewed interest in cover crops in the past fifteen years. Cover crops are used worldwide in maize cultivation, especially in the US (Brennan and Boyd, 2012; Dabney, Delgado and Reeves, 2001; Snapp et al., 2005a). Singer et al., (2007) surveyed 1,000 farmers in 2007 in the US Corn Belt (Illinois, Indiana, Iowa, and Minnesota) about cover crop use. They reported that only 18% had used cover crops in the past, 8% had cover crops in the year of the survey and on average on 6% of their land. In 2015, cover crop adoption rose to 84% of the respondents (1,200) of a questionnaire sent by Sustainable Agriculture Research and Education program and the Conservation Technology Information Centre in the USA. Today, cover crops are a central strategy of soil conservation programs to enhance physical, chemical, and biological properties of US soils (Lehman et al., 2015a).

Cover cropping is also an emerging practice in the EU (Gabriel et al., 2012; Thorup-Kristensen, Magid and Jensen, 2003). This interest is due to the multifunctionality of cover cropping, which makes them a suitable option to address the multiple threat to soil and the environment (Blanco-Canqui et al., 2015). However, the cover crop addition increases system complexity, and it is difficult to predict of possible outcomes (Teasdale et al., 2007). Peigné et al., (2015) interviewed 159 organic farmers in Europe, and the majority adopted cover crops. Their study reported that in Northern countries, organic farmers use cover crops more than in South of Europe, probably because of the lack of competition with the main crop for water.

The effect of cover crops is extremely site and species specific (Keisling et al., 1994). It varies according to climate (precipitation, temperature), soil type (texture), plant species (leguminous or not leguminous, mixture), management practices (tillage, sowing and termination techniques) (Keisling et al., 1994). For these reasons, cover crops have not always been found to improve soil quality

(Blanco-Canqui et al., 2013; Quemada et al., 2013; Welch et al., 2016; Tonitto et al., 2006). Additionally, cover cropping is often combined with other soil conservation practices such as organic agriculture, reduce tillage, organic amendments and no-till, therefore, it is difficult to isolate the effects of cover crops and to attribute the benefits on SH to the cover crops alone (Finney, Buyer and Kaye, 2017).

The UK together with other countries of Northern Europe (Denmark, Scandinavia) is part of a climatic region characterised by cold, wet winters (with temperature below zero) and warm, short summers. In these regions cover crops are traditionally grown for runoff and erosion control, limiting nutrient leaching over autumn and winter and subsequent release of these nutrients to the following cash crop (Teasdale et al., 2007).

Regardless of the multiple studies that have demonstrated soil and environmental benefits of cover cropping, the practice is not common among farmers but it has been increasing in the past 20 years (Alvarez, Steinbach and De Paepe, 2017; Martinez-Feria et al., 2016). This is partially due to the cost of cover cropping and it highlights the necessity of public subsidisation for more extensive societal benefits. For example, the majority (56%) of the respondent (1,000) of a survey sent to US maize grower in the corn belt by Singer et al., (2007) indicated that they would adopt cover crops if they would receive monetary incentives. The direct costs for the farmers are the cost of the seeds (particularly if more than one cover crop is used) and the cost of sowing and termination. Indirect costs can derive from an improper management of the cover crops that can produce a reduction of in main crop yield (Dabney et al. 2012; Snapp et al. 2005). A reduction in cost can be derived from the decrease of chemical fertilizers needed due to the incorporation of cover crops and retention of nutrients in the soil. Cover crops can also be used for grazing animals or as a source of hays (Keisling et al., 1994). Other factors are probably limiting cover crops adoption. For example, Vincent-Caboud et al., (2017) review concluded that the principal factors limiting cover crop and no-till adoption amongst organic farmers in Europe were the lack of consistent results

regarding weed control and the absence of best practices for cover crop management. They concluded their research highlighting the need for more research into in cover crop species selection and management (date of sowing/termination).

1.5.2 Effect of cover crops on yield

Undersowing cover crops may cause a reduction of in crop yield in the same way they reduce the emergence of weeds due to crop allelopathy or competition for water (Kumwenda et al., 1993) and nutrients (Kramberger et al., 2009a, 2014). For example, forage rye (*Secale cereale*) has been reported to reduce the maize yield (Iqbal et al., 2015; Pantoja et al., 2015). In dry environments crop yield is affected mainly by water availability; therefore, cover crops can compete with the primary crop for water and reduce in yield; this could be avoided by appropriate irrigation (Unger and Vigil, 1998) or rainfall (Abdin et al., 2000). For example, Martinez-Feria et al., (2016) reported a 6% reduction of in maize yield due to water competition with rye cover crop in the US.

Competition between main crop and the cover crop can be limited by periodically cutting the cover crop (Faget et al. 2012; Liedgens et al. 2004). An increase of N fertilisation could overcome the negative impact of a reduction of maize yield when competition for nutrients is a limiting factor, for example due to planting maize in a living mulch of *L. multiflorum* subsequently killed with herbicides (Garibay et al., 1997). Alternatively, cover crops are commonly killed off by exposure to frost, either using herbicides or mowing before the primary crop is planted (White and Worsham, 1990). Cover crop mulch can delay and reduce crop emergence due mechanical resistance, reduced light and soil temperature (Teasdale et al., 2007). For example, Bezuidenhout et al., (2012) found that maize emergence and growth was delayed by the presence of rye (*Secale cereale*) and ryegrass (*Lolium* sp.) residues. However, in hot-climates cover crop mulch can enhance crop emergence by retaining more water and decreasing soil temperature.

Leguminous plants have neutral or positive effects on yield. For example, Gabriel et al., (2016) reported that maize yield was not affected by ten years of barley and vetch cover cropping in Spain. The effect of cover crops on yield also depends on other soil management factors, such as tillage. Wittwer et al., (2017) reported that despite maize yield was the most significant in conventional tillage, cover crop positive effect on yield increased with the decrease of management intensity, +24% yield in no-till organic systems compared to +8% on conventional systems.

1.5.3 Cover cops for erosion protection

Cover crops can be used as an effective soil erosion control measure because they protect the soil from the direct impact of raindrops and are also able to condition soil physical properties (Langdale et al., 1991; Kaspar and Singer, 2011). Grasses are commonly used as cover crops for erosion control. They establish rapidly, protect the soil from the direct impact of raindrops, their fibrous root decreased soil erodibility, and crop stem density reduces runoff velocity and enhances sediment deposition over detachment (Burney and Edwards, 2005; Liedgens, Frossard and Richner, 2004b). The efficiency of cover crops in soil erosion control depends on the effective establishment of the cover crops before the critical period (rainiest period) and the crop biomass (Keisling et al., 1994). A mixture of cover crops can provide more canopy cover due to canopy stratification and increased biomass and, therefore, it can be more efficient than a single species (Wortman et al., 2012). Martin & Cassel, (1992) reported a 13 and 92% reduction in runoff and soil loss in a mouldboard ploughed maize system under rye (*Secale cereale*) and hairy vetch (*Vicia villosa*). Rye (*Secale cereale*) and ryegrass (*Lolium multiflorum*) have been used efficiently to reduce soil erosion in maize in Laloy and Biielders, (2010). However, many studies have reported a lack of effect on soil erosion and runoff. For example, Kaspar et al., (2001) stated that oat (*Avena sativa*) did not reduce erosion in two out of the three observed years, probably due to the high variability in runoff and soil loss measurements (see also Gómez et al., 2001; Hjermfelt and Burwell, 1984).

1.5.4 Soil cohesion, aggregate stability and water infiltration

Cover crop roots increase soil aggregate stability, soil cohesion and shear strength through the mechanical binding action of the roots (De Baets et al., 2008; De Baets et al., 2011; Perin et al., 2002), increase water infiltration and reduce soil compaction (Pratt et al., 2014). The extent of these benefits depends on the species, which determine the root characteristics such as the length and diameter (Chen and Weil, 2011a). Tap-rooted crops brassicas for instance, such as Forage radish (*Raphanus sativus*) and Rapeseed (*Brassica napus*), are more effective in increasing water infiltration and decreasing soil compaction, because they act as bio-drills, while grasses have shallow fibrous roots that reduce erosion rates to a larger extent compared to tap rooted species (De Baets et al., 2007; Chen and Weil, 2011b; Pratt et al., 2014).

Accumulation of C and SOM through the incorporation of cover crop improves the formation and stability of soil aggregates. The improved stability of the aggregates results in a better soil structure and improved water infiltration, water retention capacity, and saturated hydraulic conductivity (Keisling et al., 1994). Keisling et al., (1994) stated increased soil porosity, saturated hydraulic conductivity, and water retention capacity at sites where winter rye (*Secale cereale*), hairy vetch (*Vicia villosa*), and crimson clover (*Trifolium incarnatum*) have been cultivated for 17 years in a cotton plantation. Haruna et al., (2017) reported that winter rye (*Secale cereale*) increased macropores by 24% and increased saturated hydraulic conductivity. Basche et al., (2016) reported that after 13 years of winter rye (*Secale cereale*) in a maize-soybean rotation cover crops increased field capacity by 11% and plant available water by 22%.

1.5.5 Soil organic matter, carbon and nutrient management

Cover crops increase soil C, nutrients and OM (Carter, 2002b; Gabriel and Quemada, 2011). Their contribution to soil fertility is due to root exudates and crop residue decomposition by soil microbiology (Brennan and Boyd, 2012). Leguminous plants such as vetches (*Vicia sativa*, *Vicia villosa*) and clovers (*Trifolium repens*, *Trifolium pratense* and *Trifolium alexandrinum*) increase soil mineral nitrogen by fixing N (Brennan and Boyd, 2012). They are used to

restore soil fertility, while grasses mainly contribute to soil C. Kramberger et al. (2014a) found that crimson clover (*Trifolium incarnatum*) increased soil N supplies to the following maize.

Cover crops also increase C sequestration and SOM. Moore et al. (2014) found that after nine years of cereal rye (*Secale cereale*) cover cropping in a maize-soybean rotation SOM increased by 15%. Cover crops improve soil C by reducing C losses through soil erosion and by increasing the amount of C input into the soil by root exudates and by the incorporation of the cover crop residues (Keisling et al., 1994). In addition, OM and C can decrease due to cover cropping caused by in rhizosphere detritusphere hotspots priming effects (Nivelle et al., 2016b). The increase availability of labile organics stimulates the activity of the microbiology, which can in turn increase OM decomposition (Section 1.5.8). According to a meta-analysis by Poeplau & Don (2015) based on 30 studies that were located in different 37 sites and used 27 different species of cover crops leguminous and not leguminous, cover crops have the potential to sequester an increasing larger amount of SOC over time. Cover crops can sequester SOC with a rate of $0.32 \pm 0.08 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ of C in the first 22 cm soil, until system saturation that was predicted to be after 155 years. Therefore, the duration of the implementation is fundamental to see changes in soil C, as they are infrequently evident in the early years.

Tillage also affects SOM and C accumulation by dictating the rate of decomposition and it can obscure cover crop effects (Keisling et al., 1994). Olson et al. (2014) found that after 12 years hairy vetch (*Vicia villosa*) and cereal rye (*Secale cereale*) sequestered double the amount of C under no-till compared to chisel plough and eight times more compared to under mouldboard plough. However, Grebliunas et al., (2016) reported no significant effects of cereal rye (*Secale cereale*) and daikon radish (*Raphanus sativus* var. *Longipinnatus*) in a tilled maize production on water-extractable OC after three years, and attributed the lack of significant effects to the short term of the trial.

Cover crops can also affect GHG emissions due to changes in soil moisture and C and N availability on the soil. Sanz-Cobena et al., (2014) studied the

effect of barley (*Hordeum vulgare*), vetch (*Vicia* sp.), and forage rape (*Brassica napus*) on N₂O, CO₂ and CH₄ emissions during cover crop growth and after incorporation. They concluded that vetch (*Vicia* sp.) increased N₂O emissions by four times during cover crop growth, and barley (*Hordeum vulgare*) doubled CO₂ emission during growth. Negassa et al., (2015) reported that winter rye (*Secale cereale*) in maize increase CO₂ while N₂O emissions did not differ between cover crop and bare fallow. Kim et al., (2017), after two years of experimentation, reported an increase in N₂O emissions with the increasing incorporation of cover crop residues of barley (*Hordeum vulgare*) and hairy vetch (*Vicia villosa*) in a maize system in the Republic of Korea.

1.5.6 Groundwater protection

Cover cropping affects the quality of the groundwater. Grasses (*Lolium* sp.), small grains (*Secale cereale*, *Avena sativa*), and brassicas (*Brassica rapa*, *Raphanus sativus*) can reduce of deep percolations of nutrients, like P and N (Gabriel, Muñoz-Carpena and Quemada, 2012; Meisinger et al., 1991; Restovich, Andriulo and Portela, 2012; Salmerón et al., 2010). These non-leguminous cover crops are usually more efficient in sequestering nutrients over-winter than pure stands of leguminous plants (Brennan and Boyd, 2012) and they are known as catch crops. Catch crops are shown to uptake from 10 to 200 kg of N per hectare, depending on root development, amount of N in the soil and the above-ground biomass of the crop (Thorup-Kristensen, Magid and Jensen, 2003). Martinez-Feria et al., (2016) reported a reduction of nitrates (NO₃) losses by 20% in rye (*Secale cereale*) compared to fallow in a maize system in the US. Thomsen & Hansen, (2014) in a study in northern Europe, reported that N uptake was more abundant for winter cruciferous (Brassicaceae) than for ryegrass (*Lolium* sp.). Munkholm & Hansen, (2012) did not report a significant difference in N uptake between dyer's woad (*Isatis tinctoria*), perennial ryegrass (*Lolium perenne*) and fodder radish (*Raphanus sativus*) in 2 years long study in Denmark. Komainda et al., (2016) reported that rye (*Secale cereale*) was more effective in accumulating biomass and N than Italian ryegrass (*Lolium multiflorum*) in a maize system in Germany.

Another crucial factor affecting the effectiveness of catch crops is the sowing date. Early establishment guarantees time for growing and nutrient uptake (Teixeira et al. 2016). For instance, Thomsen & Hansen, (2014) reported a decrease 50% in N uptake when winter cover crops are sown post-harvest compared to under-sown with the main crop, due to the limited time of the experimentation. When catch crops are incorporated into the soil they can cause N deficiency because of the increased soil C/N, or they can increase soil N, depending on the C/N of the residues. These differences can result in a reduction or increase of yield (Liedgens, Frossard and Richner, 2004b). For instance, Kramberger et al. (2009) reported that Italian ryegrass (*Lolium multiflorum*) and winter rape (*Brassica napus*) incorporated into the soil decreased N and maize yield, while clovers (*Trifolium* sp.) increased N and maize yield, due to the low C/N of the residues.

1.5.7 Weed and pest control

Herbicides are often used as weed control measure in the majority of the agricultural systems, as well as in maize systems (Mhlanga, Chauhan and Thierfelder, 2016). Cover crops can be used as alternative weed control measure, particularly, in no-till organic cultivations. They can influence weed population either alive or in the form of mulch (Teasdale et al., 2007). Cover crops suppress weed germination by increasing competition for available light (Teasdale and Mohler, 2000), nutrients (Uchino et al., 2011, 2012) and by releasing allelopathic substance (Brust, Claupein and Gerhards, 2014). However, the effectiveness varies (Mhlanga et al., 2015). The success of weed control depends on the cover crop selected, their vigour and the season during which are grown (Teasdale et al., 2007). Cover crops adapted to warm environments, successfully suppress weeds during the summer fallow, e.g. velvet bean (*Mucuna pruriens*), jack bean (*Canavalia ensiformis*), cowpea (*Vigna unguiculata*), sorghum (*Sorghum bicolor*) (Teasdale et al., 2007). For example, Mhlanga et al., (2015) observed 92% reduction in weed density in maize-velvet bean and maize- jack bean rotations but high weed densities in black sunn hemp (*Crotalaria juncea*) and cowpea (*Vigna unguiculata*). Choudhary et al.,

(2014), reported a decrease of weed density in a maize-cowpea intercropping system. Jamshidi et al., (2013) reported that cowpea reduced by 45% weed density when intercropped with maize. Yeganehpour et al., (2014) observed that clover (*Trifolium* sp.) increased maize yield the most and had the less weed density compared to hairy vetch (*Vicia villosa*), basil (*Ocimum basilicum*) and dill (*Anethum graveolens*) in Iran. Cover crops adapted to cool environments, such as rye (*Secale cereale*), hairy vetch (*Vicia villosa*), clovers (*Trifolium* sp.) perform better as winter cover crops than as summer ones and, therefore, they can have a limited effect on weeds in summer (Teasdale et al., 2007). Cover crops that are winter killed such as mustard (*Brassica* sp.) suppress weed effectively during the winter months but less during spring (Teasdale et al., 2007). Uchino et al. (2012) and Uchino et al. (2009) found that winter rye (*Secale cereale*) and hairy vetch (*Vicia villosa*) reduction of weed density in maize during autumn and winter.

Cover crops can also act as pests control, because they can host predatory insects and spiders (Rämert 1996; Feil et al. 1997) and can enhance soil biodiversity, by providing a more resources and shelter for above-ground (insects) and belowground biota (soil microorganisms, earthworms). Cover crops can also control pests of the following crop. For example, Lundgren & Fergen (2010) found that a grass cover crop (*Elymus trachycaulus*), planted in autumn, increased the abundance of predators of western corn rootworm (*Diabrotica virgifera virgifera*), reduced the number of third instars (young arthropods), and reduced root damage in maize that followed on. However, the positive effects on soil biota can be neutralised by the use of herbicides or mowing to kill the cover crop off (Fox et al., 2016). Fox et al., (2016) reported that adding a cereal rye (*Secale cereale*) and Austrian winter pea (*Pisum sativum* subsp. *arvense*) did not increase arthropod predators in maize in the US, due to the removal of the cover crops biomass in spring.

1.5.8 Effect on soil organisms

A complete understanding of cover crop species-specific effects on soil organisms is still missing, and investigations on cover crop effects on

organisms often involve other management practices such as tillage and cover crop residue incorporation (Finney, Buyer and Kaye, 2017). Additionally, despite a better understanding today of the effects of cover cropping on soil microbiology, there is no complete understanding of how microbial characteristics affect specific soil functions (Finney, Buyer and Kaye, 2017).

Cover crops can have a positive effect on soil microbiology due to an increase in SOM, roots exudates and by creating a more suitable environment for life (e.g. water, soil structure, temperature) (Nivelle et al., 2016b). Factors controlling the magnitude of the effects are the duration and the cover crop species involved. Additionally, climate plays an important role, and in semi-arid environments cover crops benefits on soil biota are limited (Calderón et al., 2016).

Balota et al., (2014) reported an increase in MB, labile polysaccharides, easily extractable glomalin-related soil proteins and enzyme activity after 23 years of cover cropping in a maize-soybean rotation using five different winter cover crops: black oats (*Avena strigosa*), wheat (*Triticum aestivum*), radish (*Raphanus raphanistrum* subsp. *sativus*), blue lupin (*Lupinus angustifolius*), and hairy vetch (*Vicia villosa*). The microbial community of the conventionally managed treatments was more enhanced by the cover crops compared to no-till treatments, due to the lower initial state of the microbial community in the conventional treatments compared to the no-till.

Leguminous cover crops usually have more significant effects than grasses due to an increased N availability (Mbuthia, 2014). Kirchner et al. (1993) found an enhanced MB and soil enzyme activities during crimson clover (*Trifolium incarnatum*) growth in conventional maize. Italian ryegrass (*Lolium multiflorum*) and a mixture of winter rye (*Secale cereale*) and oat (*Avena sativa*) increased MB after two years in no-till maize (Faé et al. 2009). Nivelle et al., (2016b) reported that after five years under no-till and no N fertilisation, two mixtures of cover crops, one with a low prevalence of legumes and one with a high incidence of legumes, had higher microbial activity compared to bare fallow. However, this difference did not subsist under N fertilisation, which probably

stimulated microbial activity even in the plots without cover crops. Finney et al., (2017) reported that eight cover crops species (sunn hemp *Crotalaria juncea*, soybean *Glycine max*, red clover *Trifolium pratense*, hairy vetch *Vicia villosa*, forage radish *Raphanus sativus*, oat *Avena sativa*, rapeseed *Brassica napus*, cereal rye *Secale cereale*) increased microbial biomass after two months from planting. Additionally, plant biomass was correlated to MB in this study.

Cover crops can also affect the structure of the microbial community. Verzeaux et al., (2016a) reported that, after 4 years of experimentation in France, the incorporation of a mixture of Egyptian clover (*Trifolium alexandrinum*), fava bean (*Vicia faba*), vetch (*Vicia sativa*), flax (*Linum usitatissimum*), phacelia (*Phacelia tanacetifolia*) and oats (*Avena sativa*) prevented the adverse effect of fertilizers on the diversity of soil microbial community. Fernandez et al. (2016) reported that after two years of cover cropping, cover crop incorporation had mixed effects on microbial diversity in maize. It decreased immediately after the incorporation of in winter rye (*Secale cereale*), oilseed radish (*Raphanus sativus* var. *oleifer*), and buckwheat (*Fagopyrum esculentum*), but increased later in the season compared to the control. Finney et al., (2017) found that alive cover crops (sunn hemp *Crotalaria juncea*, soybean *Glycine max*, red clover *Trifolium pratense*, hairy vetch *Vicia villosa*, forage radish *Raphanus sativus*, oat *Avena sativa*, rapeseed *Brassica napus*, cereal rye *Secale cereale*) increased Gram-negative bacteria, non-AM fungi and AM fungi. Chavarría et al., (2016) reported an increase of Gram-positive bacteria at cash crop harvest in a no-till system after only two years of cover crops (a mixture including *Vicia villosa*), due to the increased residues after the incorporation of the cover crops. The increase in MB can also be due to an increase in AMF (Lehman et al., 2014). For example, *Vicia villosa* grown over winter increased the inoculum of AMF in soil (Galvez et al., 1995). Njeru et al., (2014) reported that hairy vetch (*Vicia villosa*) increased mycorrhizal colonisation in maize plants of 35% compared to the natural vegetation (agricultural weeds). However, Indian mustard (*Brassica juncea*) decreased the potential of soil mycorrhization caused by the release of isothiocyanates, after the incorporation of the mustard

residues. Lehman et al., (2012) reported an increase of the mycorrhizal inoculum potential after oats (*Avena sativa*).

Cover crops usually increase fungal biomass. The incorporation of *Vicia villosa* was found to increase fungal biomass (ergosterol) from 5 days after residue incorporation and increased PLFA fung/bacteria markers, due to the cellulosic nature of the vetch residues, which are more available to fungi than to bacteria (Lucas, Angelo and Williams, 2014). Calderón et al. 2016 reported an increase in PLFA fungal markers (18:1 ω 9c, 18:2 ω 6c and 18:3 ω 6c) after oat (*Avena sativa*), pea (*Pisum sativum*), flax (*Linum usitatissimum*) and rapeseed (*Brassica napus*) termination compared to bare soil in no-till wheat. Kataoka et al., (2017), as well, found an increase in fungal biomass (ergosterol) due to vetch (*Vicia villosa*) residues incorporation.

1.6 Aim, objectives and hypotheses

This research aims to investigate the role that four species of cover crops have towards enhancing soil microbial community and their role in preventing soil erosion and runoff during maize production.

Research Objectives:

1. Quantify cover crop/soil microbiology interactions in a maize production.
2. Critically evaluate and quantify the role of different species of cover crops towards reducing surface runoff and soil loss compared to standard post-harvest management practices.
3. Use information gained to evaluate the viability and practicability of using cover crops for maize production.

These objectives will be achieved over the course of two field experiments devised to answer the following hypotheses.

1.6.1 Overarching hypothesis

Planting cover crops during maize production will affect soil microbial community, reduce runoff, and control soil erosion compared to various conventional post-harvest methods.

1.6.2 Experiment 1 specific hypotheses

1. Test the overarching hypothesis using *Lolium multiflorum* and *Secale cereale* as cover crops.

1.6.3 Experiment 2 specific hypotheses

1. Test the overarching hypothesis using the cover crop *Lolium multiflorum* and the cover crops mixtures *Lolium multiflorum* & *Trifolium alexandrinum* and *L. multiflorum* & *Vicia villosa*.
2. The cover crops will increase maize yield compared to the post-harvest standard practice without cover crops.
3. Cover crop mixtures will have a more significant effect on soil microbiology than *Lolium multiflorum* alone.

2 METHODOLOGY

2.1 Catchment aspect

The field experiments were located in the Lugg sub-catchment, which is part of the Wye catchment, located across the border between Wales and England. The north and west part of the Wye catchment is primarily livestock, while the south and the east part (river Lugg sub-catchment) is mostly intensive arable farming. The rivers Wye and the Lugg are classified as Sites of Special Scientific Interest and parts of the river Lugg and the river Wye are classified as Special Areas of Conservation (SAC) under the European Community Habitats Directive. Phosphate levels of the SAC are exceeding the target levels for those areas due to sediments (Jarvie et al., 2005). A nutrient management plan has been devised to address this problem. One of the focal points of the River Wye SAC Nutrient Management Plan is the reduction of soil erosion and the prevention of phosphate contamination via runoff from arable land, of which forage maize fields are of particular concern (Environment Agency and Natural England, 2014).

2.2 Field site characteristics

This research comprised of two field experiments that were conducted within the same field. Experiment 1 ran from November 2014 (after the harvest of maize) to March 2015 (before cover crop termination), Experiment 2 from August 2015 (after maize germination) to March 2016 (before cover crop termination). The experiments were located within commercially grown maize (Herefordshire, UK: OS grid references: 344505 E, 257646 N). Annual average rainfall for the area was 793.8 mm yr⁻¹ (MetOffice, 2016a). Soil was classified as the Bromyard Soil Association (National Soil Resources Institute, 2017). This typically consists of well-drained reddish fine silty soils over shale and siltstone. Soil texture measured in the field was a sandy silt loam according to the UK soil textural triangle (Hodgson et al., 1976). Slopes ranged from three to four degrees.

2.2.1 Weather data

Weather data was monitored throughout the field trials using the Ross-On-Wye weather station (Herefordshire, UK: OS grid references: 359800E 223800N), which is 30 miles away from the field experiment (Table 3). In addition, on-site weather data was collected via a weather station equipped with DT80/2 data logger located within the experimental field (CWi Technical Ltd; Table 4). Data capture was triggered by a 2mm (min) rainfall event. A GSM/GPRS (Global Systems for Mobile Communications/General Packet Radio Service) modem attached to the data logger allowed either GSM data pull or GPRS data push to a web server. The data logger was solar powered and maintained with an 80 Ah professional gel cell linked to a 50 W panel.

Table 3 Weather data (average) collected by Ross-On-Wye weather station (MetOffice, 2016b) for the duration of Experiments 1 and 2.

Exp. [†]	Year	Month	Max. Temp. [‡] (°C)	Min. Temp. [§] (°C)	Air frost (days)	Rainfall (mm)	Sun (h)
1	2014	11	11.4	5.8	4	113	53.3
1	2014	12	9.1	2	13	35.2	70.7
1	2015	1	8.5	2	7	73	90.9
1	2015	2	7.8	1.2	12	35.3	86.1
1	2015	3	11	3.4	4	38.4	119.9
2	2015	8	20.5	12.2	0	92.5	145.4
2	2015	9	18.2	8	0	36.3	171.4
2	2015	10	15.3	7.5	0	38	78
2	2015	11	12.9	7.4	3	65.7	25.9
2	2015	12	13.1	8.3	0	125.9	24.9
2	2016	1	9.2	3.1	5	115.4	48.2
2	2016	2	9.1	2.1	7	79.5	96.5
2	2016	3	10.4	1.9	8	68	124.3

†=Experiment, ‡= Maximum Temperature, §=Minimum Temperature

Table 4 Weather data collected on site for the duration of Experiments 1 and 2.

Collection period	Rainfall	Numb. Of days[§]	Average Temp. [†]
	(mm)		(°C)
November (5-30) 2014	73	21	7
December (1-31) 2014	19.6	14	-
January (1-31) 2015	72	26	-
February (1-28) 2015	35.6	16	4
March (1-15) 2015	23	9	6
August (6-14 20-31) 2015	78	11	12
September (1-30) 2015	21	22	12
October (1-13) 2015	1	7	10
November 2015a	-	-	-
December (21-31)2015	56	11	10
January (1-2 13-30) 2016	34	16	5
February (16-29) 2016	12	6	5
March (1-15) 2016	33	10	5

†= Average Temperature, §= Number of days, - = data not collected due to battery failure.

2.2.2 Field topography

Filed topography was investigated by taking elevation point measurements every 7-10 m with a Trimble GeoXH, which is equipped with real-time kinematic corrections and a horizontal and vertical precision of <3 cm. Analysis of the topographic properties of the field was carried out using ArcGIS software (ESRI), version 10.2. A surface elevation raster with a resolution of 3.0 m was extrapolated from the survey points using an Inverse Distance Weighted

interpolation technique. A resolution of 3.0 m was deemed suitable for the surface raster based on the original point measurement resolution and to maintain micro-topographic features of interest within the field. Using the Slope tool a raster representing the slope with 3 m resolution was created from the surface elevation raster (Figure 1).

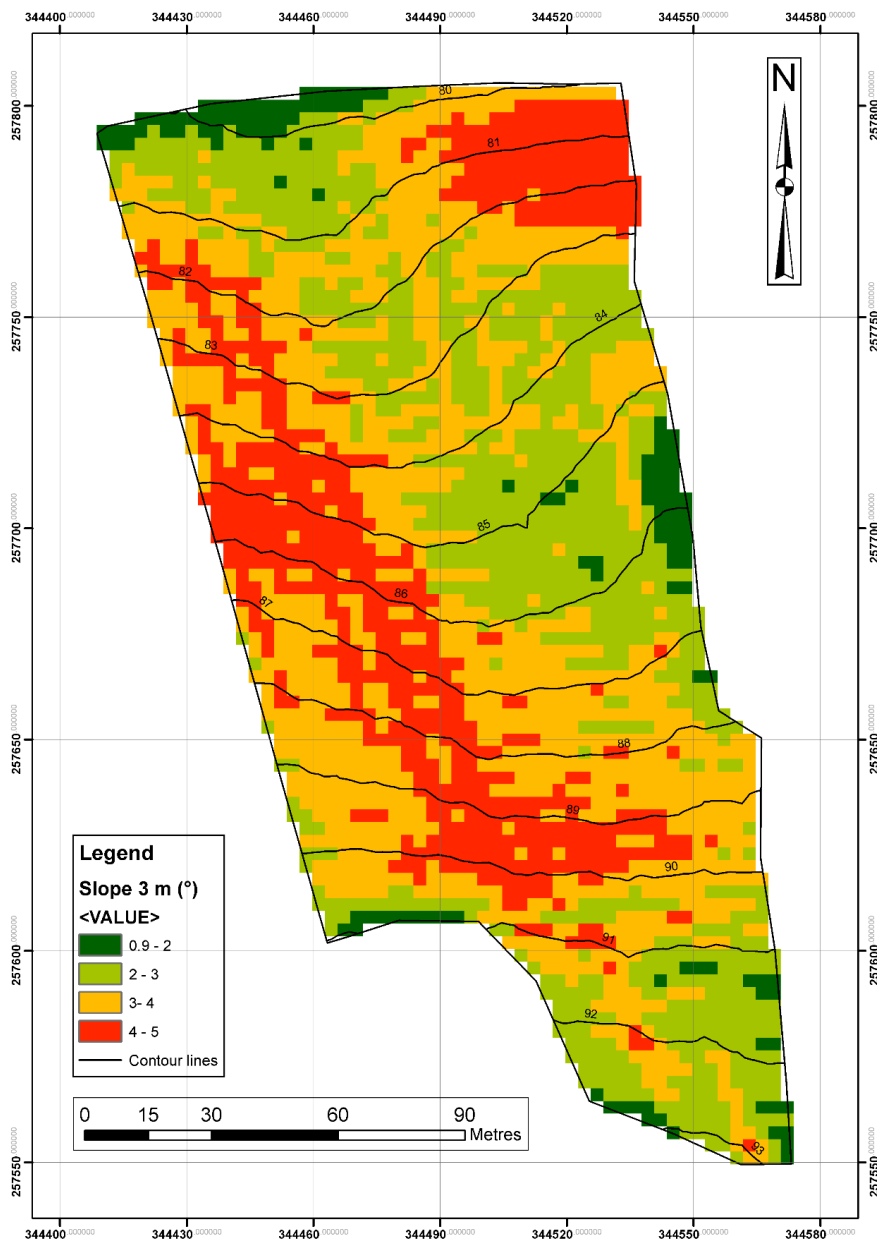


Figure 1 Map representing the slope of the field with a resolution of 3 m. The top of the map is the bottom of the slope.

2.2.3 Experimental setup

Runoff and sediments were collected from fully instrumented erosion plots (Figure 2). The erosion plot design remained consistent between the two experiments. The erosion plots were 12 m x 1.5 m, hydrologically isolated from field runoff by soffit boards dug into the soil (to a depth of Ca. 0.2 m) on three sides. During Experiment 1 plots shared no borders (Figures 2 and 3), while in Experiment 2 plots shared the intermediate border (Figure 4). Runoff and sediments were collected by a Gerlach trough placed at the lowest elevation of the plots (Figure 5), connected with a pipe to a water tank (Figures 6 and 7). Each water tank had a pre-calibrated linear level sensor (Figure 8) monitoring the water level every 30 minutes, every minute with > 0.2 mm of rainfall. The sensors and tipping bucket rain gauge were connected via a DT80/2 data logger with the weather station (Figure 9). The tanks were manually sampled to collect the runoff sediments that had accumulated. The placement of the tanks differed between the two experiments; during Experiment 1, tanks were dug into the soil (Figure 3); however, this solution was not successful and severe intervention of maintenance where necessary to stabilise the tanks (Figure 5). During experiment 2, tanks were kept on the soil surface placed on bricks, held in place by two ground anchors and rope (Figure 8).



Figure 2 Erosion plots during Experiment 1.



Figure 3 Erosion plots of Experiment 1, particular of the hole to place the tank.



Figure 4 Erosion plots of Experiment 2.



Figure 5 Particular the stabilisation of a tank during Experiment 1.



Figure 6 Particular of the Gerlach trough used to collect runoff during the experiments.



Figure 7 Particular of tanks during Experiment 2



Figure 8 Particular of the linear level sensor land of the ground anchor use to stabilise the tanks during Experiment 2.



Figure 9 Weather station used during the two experiments.

2.3 Experiment 1

Experiment 1 was devised to test the hypothesis using *Lolium multiflorum* and *Secale cereale* as cover crops during maize production would affect soil microbial community, reduce runoff, and control soil erosion compared to various conventional post-harvest methods.

2.3.1 Experimental design

Experimental plots were established in May 2014 (Table 5) and run until March 2015 when the cover crops were sprayed and incorporated; they were 12 m wide (12 maize rows) and ran for 180 m approximately. Six different treatments

were selected, three treatments included cover cropping and three treatments involved other management practices. Three erosion plots were placed inside each experimental plot (pseudo replicates).

The treatments (pseudo replicates n=3) included:

1. Post-harvest wintered maize stubble without cover crops; beforehand the area was tilled in spring with mouldboard plough prior maize drilling. Referred to as the **traditional method**.
2. Post-harvest wintered maize stubble without cover crops; beforehand the area was tilled in spring with disc plough prior maize drilling. Referred to as the **disc method**.
3. Post-harvest wintered maize stubbles without cover crops, combined with 3 m wide strips tilled post-harvest with chisel plough; beforehand, the area was tilled in spring with mouldboard plough prior maize drilling. Referred to as the **cross-plough method**.
4. *Lolium multiflorum* under-sown broadcast six weeks after maize drilling; beforehand, the area was tilled in spring with mouldboard plough prior maize drilling. Referred to as ***L. multiflorum* broadcast**.
5. *Lolium multiflorum* under-sown directly drilled and raked six weeks after maize drilling; beforehand, the area was tilled in spring with mouldboard plough prior maize drilling. Referred to as ***L. multiflorum* drilled**.
6. *Secale cereale* sown directly drilled post-harvest; spring tilled with mouldboard plough prior maize drilling. Referred to as ***S. cereale***.

Table 5 Relevant agronomical practices during Experiment 1.

Event	Date
Field tilled with mouldboard plough for treatments 1, 3, 4, 5 and 6 and with a disc plough for treatment 2	4/2014
Maize drilling with a 75 cm row spacing (Kentaurus and Sergio varieties)	15/5/2014
Application of the post-emergence herbicide Callisto	17/6/2014

Event	Date
Under-sow of Italian ryegrass (<i>Lolium multiflorum</i>) directly drilled for treatment 5 and broadcast for treatment 4 at 20 kg ha ⁻¹)	23/6/2014
Plot ploughed with chisel plough across the slope in strips (3 m wide) for treatment 3	24/10/2014
Forage rye (<i>Secale cereale</i>) drilled at 130 kg ha ⁻¹ for treatment 6.	25/10/2014
Termination of cover crops with Roundup herbicide and incorporation with mouldboard plough	14/4/2015

2.3.2 Erosion plots installation

The erosion plots were installed and operating by the 5/11/2014. Three erosion plots were placed in each experimental plot on a similar slope and in areas without signs of compaction (wheel tracks). This was carried out visually selecting the areas with a similar slope on ArcGIS (Figure 10) and by field assessment. Nevertheless, the slope was significantly different between the treatments (Table 6). The cross-plough method had the most significant slope angle, which was not more than 1.3 ° larger than the other treatments.

Table 6 Slopes for each treatment of Experiment 1, derived from the surface elevation raster. Letters represent homogenous groupings.

Erosion plot	Slope (° ± SE)
Traditional method	3.7 ^b ± 0.18
Disc method	2.9 ^a ± 0.04
Cross-plough method	4.2 ^c ± 0.11
<i>L. multiflorum</i> broadcast	2.9 ^a ± 0.01

Erosion plot**Slope****(° ± SE)***L. multiflorum* drilled2.9^a ± 0.08*S. cereale*3.7^b ± 0.04

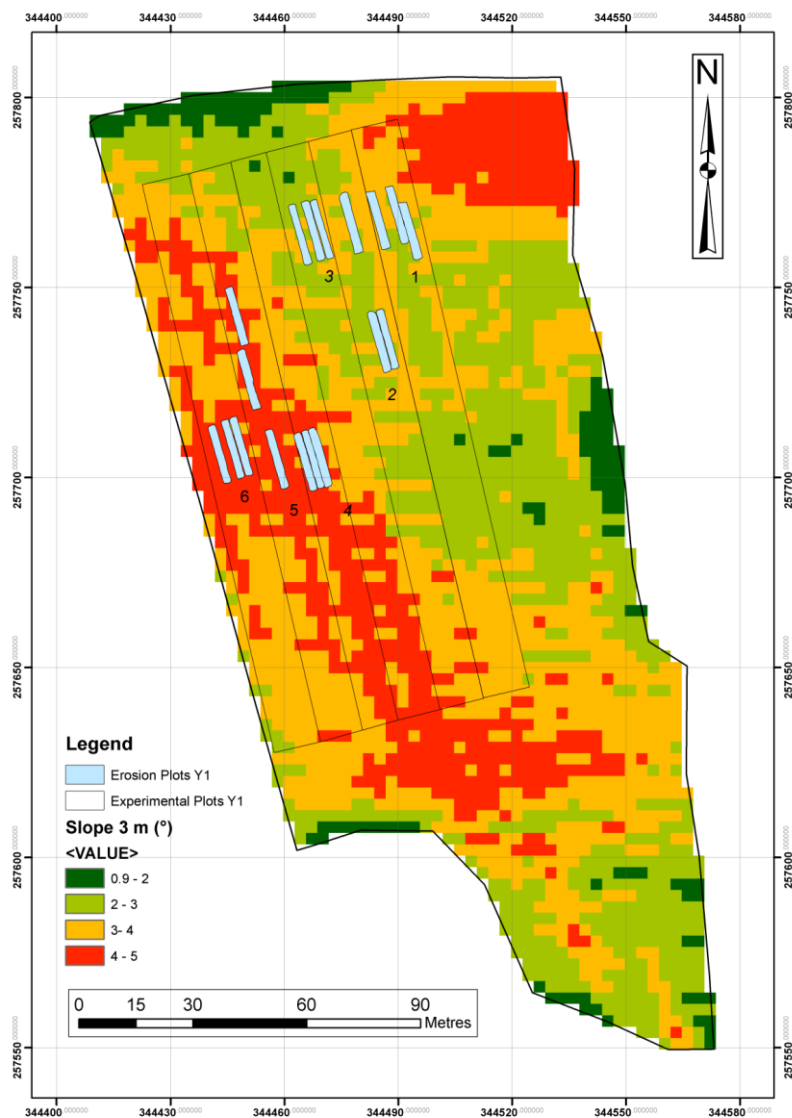


Figure 10 Experimental setup of Experiment 1. The top of the map is the bottom of the slope. 1= Traditional method. 2= Disc method. 3= Cross-plough method. 4= *L. multiflorum* broadcast. 5= *L. multiflorum* drilled. 6= *S. cereale*.

2.4 Experiment 2

Experiment 2 was devised to test the hypothesis that using *Lolium multiflorum* cover crop and the cover crops mixtures *Lolium multiflorum* & *Trifolium alexandrinum* and *L. multiflorum* & *Vicia villosa* during maize production would affect soil microbial community, reduce runoff, and control soil erosion compared to various conventional post-harvest methods. Additionally, it was hypothesised that the cover crops would increase maize yield and the cover crop mixtures would have a more significant effect on soil microbiology than *Lolium multiflorum* alone.

2.4.1 Experimental design

Experiment 2 was established in the same field of Experiment 1 after cover crop incorporation. It ran for eight months (7/2015 - 3/2016; Table 7). The experimental design consisted of four treatment variables with five replicates arranged randomly across the slope (Figure 11).

The treatment replicates (n=5) included:

1. Post-harvest wintered maize stubble without cover crops; beforehand the area was tilled in spring with mouldboard plough. Referred to as the **traditional method**.
2. *Lolium multiflorum* under-sown broadcast six weeks after maize drilling; beforehand the area was tilled in spring with mouldboard plough. Referred to as ***L. multiflorum***.
3. *Lolium multiflorum* and *Trifolium alexandrinum* under-sown broadcast six weeks after maize drilling; beforehand the area was tilled in spring with mouldboard plough. Referred to as ***L. multiflorum* & *T. alexandrinum***.
4. *Lolium multiflorum* and *Vicia villosa* under-sown broadcast six weeks after maize drilling; beforehand the area was tilled in spring with mouldboard plough. Referred to as ***L. multiflorum* & *V. villosa***.

Table 7 Relevant agronomical practices during Experiment 2.

Event	Date
Termination of previous cover crops (Experiment 1) with Roundup herbicide and incorporated in the soil	14/4/2015
Application of 8 T h ⁻¹ of chicken manure	21/4/2015
Maize drilling with a 75 cm row spacing (Sergio & Kentaurus varieties)	13/5/2015
Use of herbicides (Nico Pro 0.5 L ha ⁻¹ and Callisto 0.65 L ha ⁻¹). Erosion plots were covered with an impermeable layer to prevent the herbicides affecting cover crop establishment	11/6/2015
Cover crop mixtures seeded broadcast: i) <i>L. multiflorum</i> 56 kg ha ⁻¹ ii) <i>L. multiflorum</i> & <i>V. villosa</i> 56 kg ha ⁻¹ and 62 kg ha ⁻¹ iii) <i>L. multiflorum</i> & <i>T. alexandrinum</i> 56 kg ha ⁻¹ and 59 kg ha ⁻¹ .	25/6/2015
The seed rates were increased compared to the conventional rates to ensure cover crop establishment.	
Erosion plots operational	27/7/2015
The erosion plots dismantled for the harvest.	
During the harvest, harvester trailers were driven on the plots leaving tires marks (wheel tracks) running across the slope	29/10/2015
Erosion plots rebuilt	27/11/2015
Experiment decommissioned	15/3/2016
Cover crop terminated with Roundup herbicide	30/3/2016

2.4.2 Erosion plots installation

Experiment 2 used the same experimental setup as Experiment 1 (Section 2.3.2). However, experimental plots were located in a different part of the field (Figure 11).

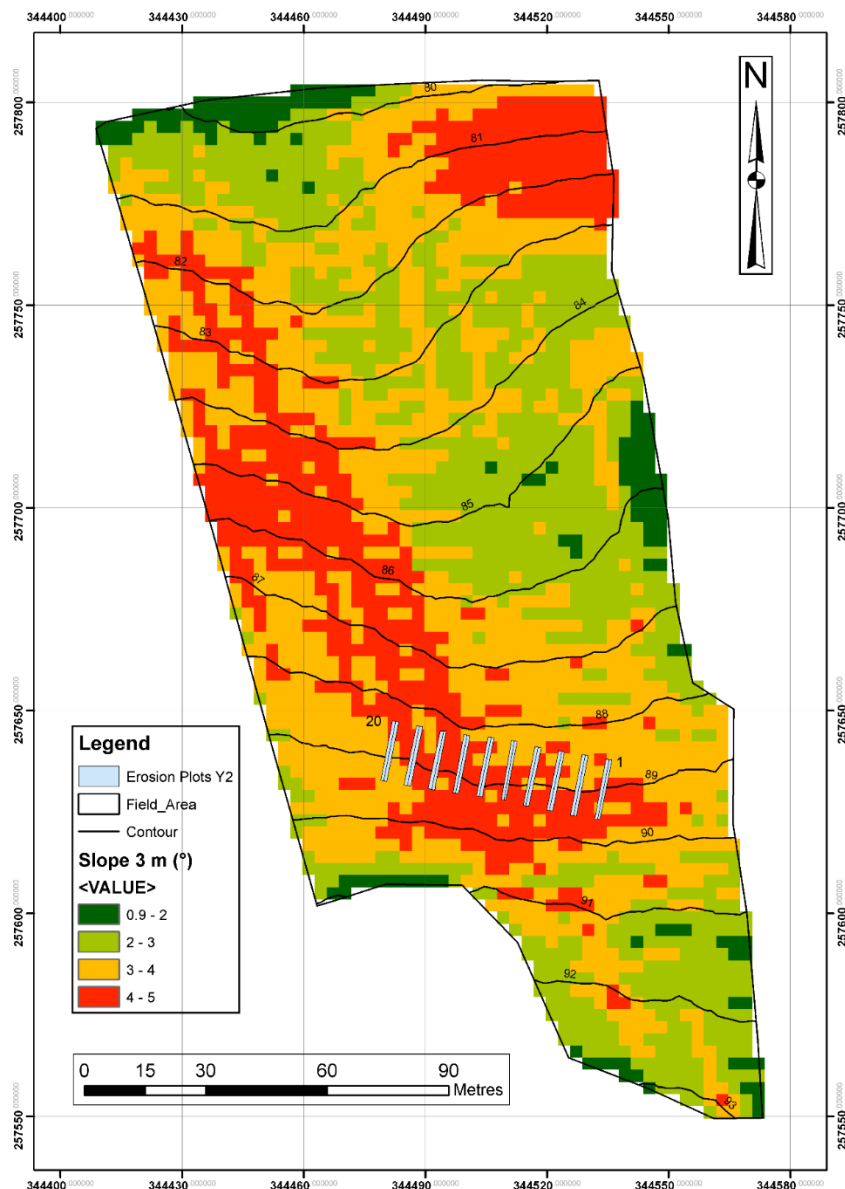


Figure 11 Experimental setup of Experiment 2. 1, 20 indicate the numbering of the erosion plots.

In Experiment 2 the erosion plots had to be removed for the harvest. During the harvest, the experimental areas were driven across leaving wheel tracks in the erosion plots. Subsequently, wheel tracks could have had an impact on runoff and soil erosion from these plots. Therefore, the number of wheel tracks in each plot was recorded, and the total volume was estimated by measuring the width and depth of 3 random tracks for each plot. The number of wheel tracks was significantly different between the treatments ($P < 0.05$; Table 8) *L. multiflorum* & *T. alexandrinum* had the most significant number of wheel tracks. However, the total volume was not significantly different ($P > 0.05$; Table 8). For this reason, it was assumed that the wheel tracks affected all the treatments in the same way and consequently did not compromise the integrity of the results.

Table 8 Number and volume of wheel tracks measured for each treatment. Letter represents homogenous groupings.

Treatment	Wheel tracks	Total volume of wheel tracks
	(Number $\pm$ SE)	(m ³ $\pm$ SE)
Control	7 ^a $\pm$ 0.3	0.1 ^a $\pm$ 0.01
<i>L. multiflorum</i>	6.4 ^a $\pm$ 0.4	0.09 ^a $\pm$ 0.01
<i>L. multiflorum</i> & <i>T. alexandrinum</i>	5.2 ^b $\pm$ 0.3	0.17 ^a $\pm$ 0.03
<i>L. multiflorum</i> & <i>V. villosa</i>	6.4 ^a $\pm$ 0.4	0.1 ^a $\pm$ 0.01

Additionally, it was noticed from the contour lines and visual inspection, that the field presented a slight depression in the middle of the plot line. For this reason, plots were additionally divided into three different areas labelled as A, B and C (Figures 12 and 13). Slopes for each erosion plot were measured (Table 9). No significant differences were found between the treatments ($P > 0.05$).



Figure 12 Erosion plots during Experiment 2 after maize harvest.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
§	§	§	†	‡	#	§	#	‡	#	†	‡	†	‡	#	#	‡	†	†	§
AREA A								AREA B						AREA C					

Figure 13 Schematic representations of the erosion plots of Experiment 2 divided into three areas A, B and C. The number represents the plot number. ‡= *L. multiflorum*, # = *L. multiflorum* & *V. villosa*, § = *L. multiflorum* & *T. alexandrinum*, † = Traditional method. A = west plots; B = central plots (depression); C = east plots.

Table 9 Slopes for each treatment derived from the Surface Elevation Raster. Letters represent homogenous groupings.

Treatment	Slope
	(Average °± SE)
Control	4.5 ^a ± 0.1
<i>L. multiflorum</i>	4.4 ^a ± 0.1
<i>L. multiflorum</i> & <i>T. alexandrinum</i>	4.2 ^a ± 0.1
<i>L. multiflorum</i> & <i>V. villosa</i>	4.4 ^a ± 0.1

2.5 Survey among UK maize growers

An online survey was devised, along with another PhD student (Alexandra Cooke), using Qualtrics (Qualtrics LLC) and subsequently sent to the Maize Growers Association with the aim of understanding how and why growers use cover crops (Appendix A). Survey results were anonymous and were summaries by statistics and graphs presented in the result section 3.1. The survey comprehended six multiple choice questions, two rank order questions and three dichotomous questions. Questions covered topics including soil erosion in maize and soil erosion control techniques, cover crop adoption and cover crop species preference.

2.6 Runoff flow analysis

Due to the division of the erosion plots in three areas in the second experimental year, the software ArcGIS (Esri) was used to analyse in more detailed the topography of the three areas. A raster representing the direction the runoff flow was created from the surface raster of the elevation (3 m resolution; Section 2.2.2) using the *flow direction* tool (Figure 14). This tool assigns to each cell of the raster one of the eight cardinal directions (N, NE, E, SE, S, SW, W, and NW), denoting the direction of surface flow from that grid cell to the steepest downslope neighbour cell.

The flow direction raster was converted to a polygon feature. Each one of the polygons in the feature had in the attribute table a value for its surface area (m^2) and a value for the direction of the flow (Figure 15). Using the *intersect* tool the flow direction polygon was cut in the shape of the single plots. The resulting plots were divided into polygons associated with a different direction of the flow (see polygons in blue for an example; Figure 16).

The area (%) of each flow direction class (N, NE, E, SE, S, SW, W, and NW) within the plots was calculated. Total area extent of each flow direction class was compared between treatments and between the areas (Tables 10 and 11). Runoff flow direction was not significantly different between treatments ($P>0.05$), but there was a significant difference ($P<0.05$) between areas. Flow direction NW in area C was 200% smaller than in area A. Flow direction N in C was 53% smaller compared to A and 43% smaller compared to B. The flow direction NE in area C was 193% larger compared to A and 108% compared to B. These results confirmed the presence of a depression in the area occupied by area B, which was observed in-field.

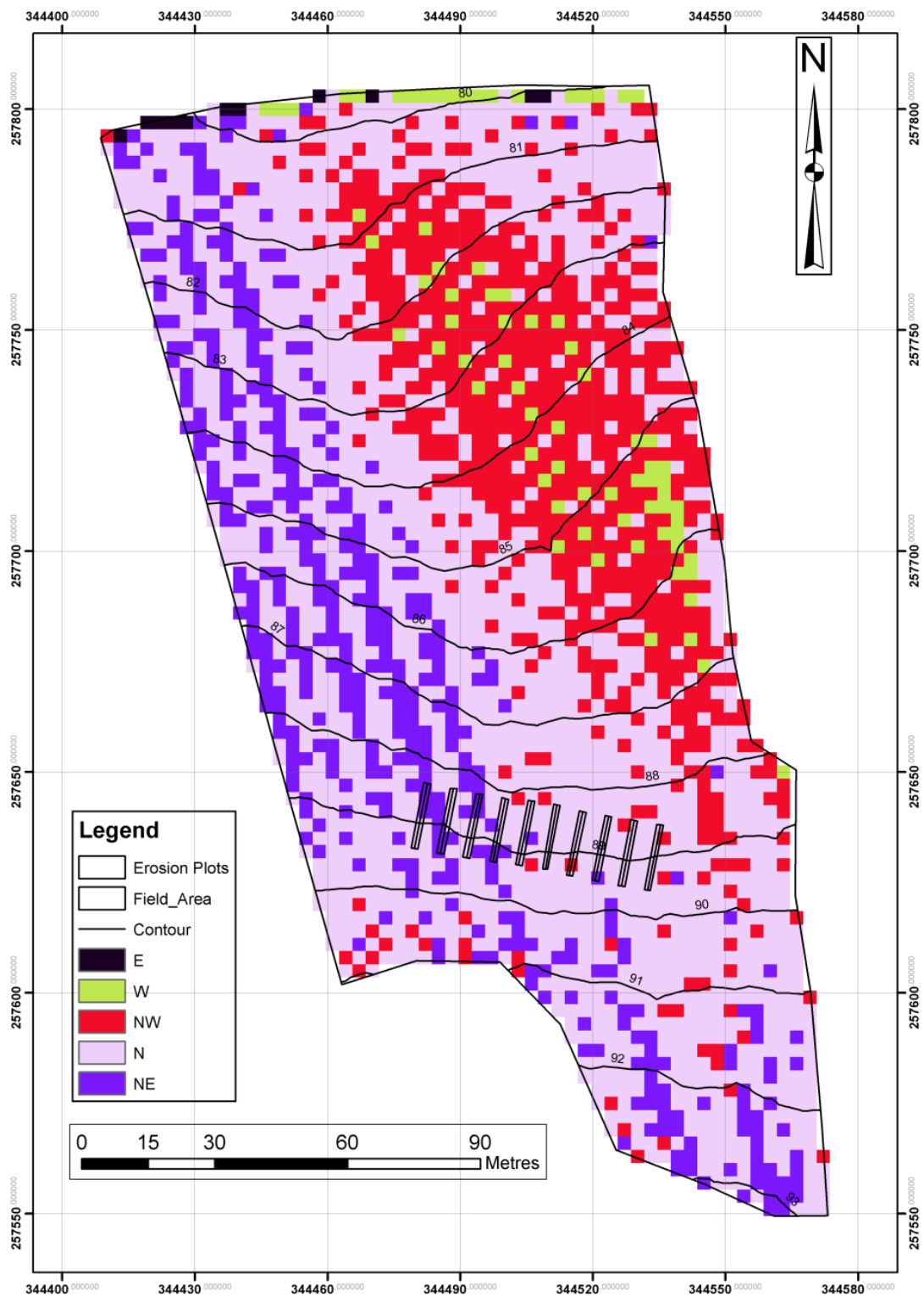


Figure 14 Map representing the Flow Direction Raster.

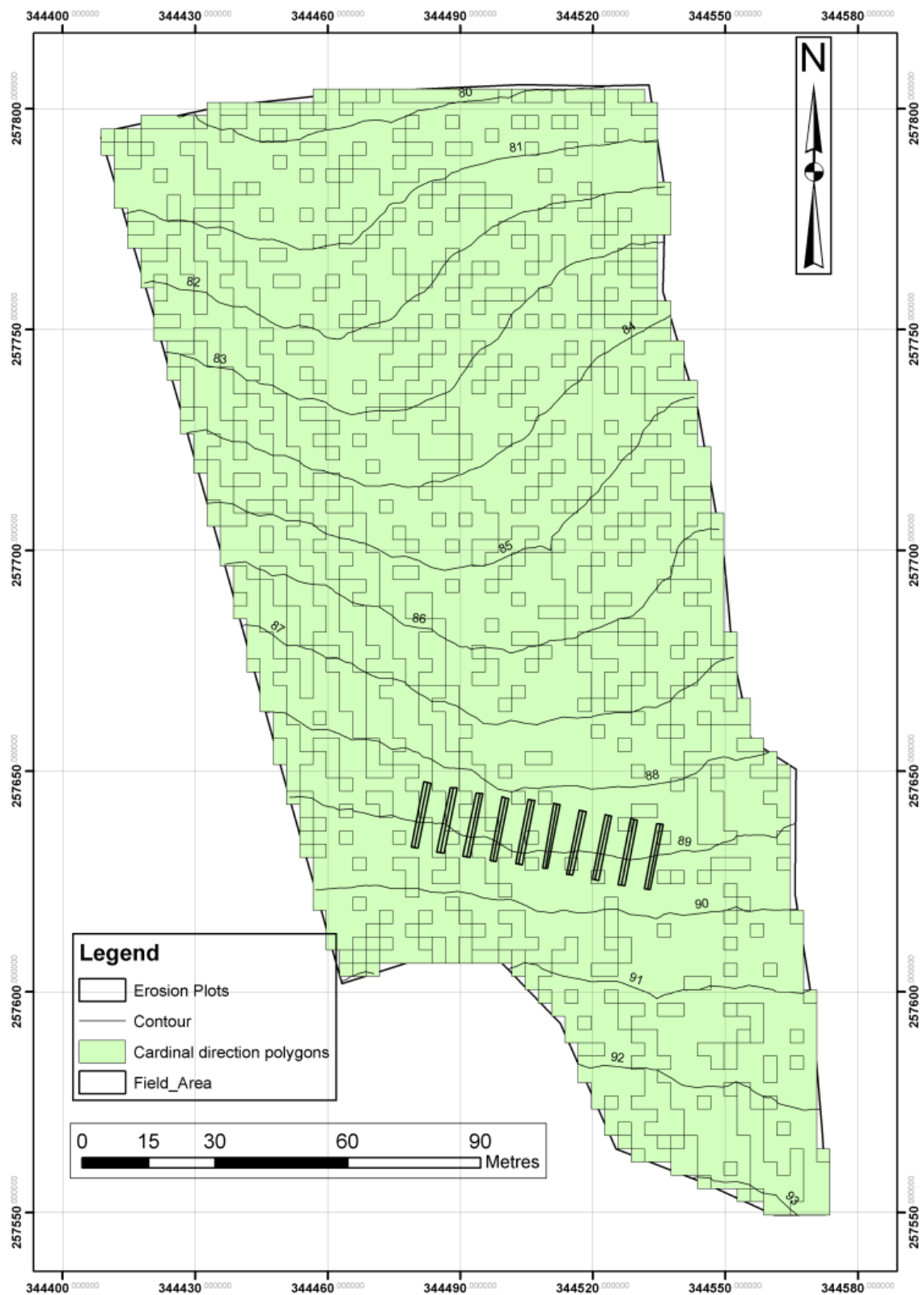


Figure 15 Map representing the Flow Direction Raster converted into polygon feature.

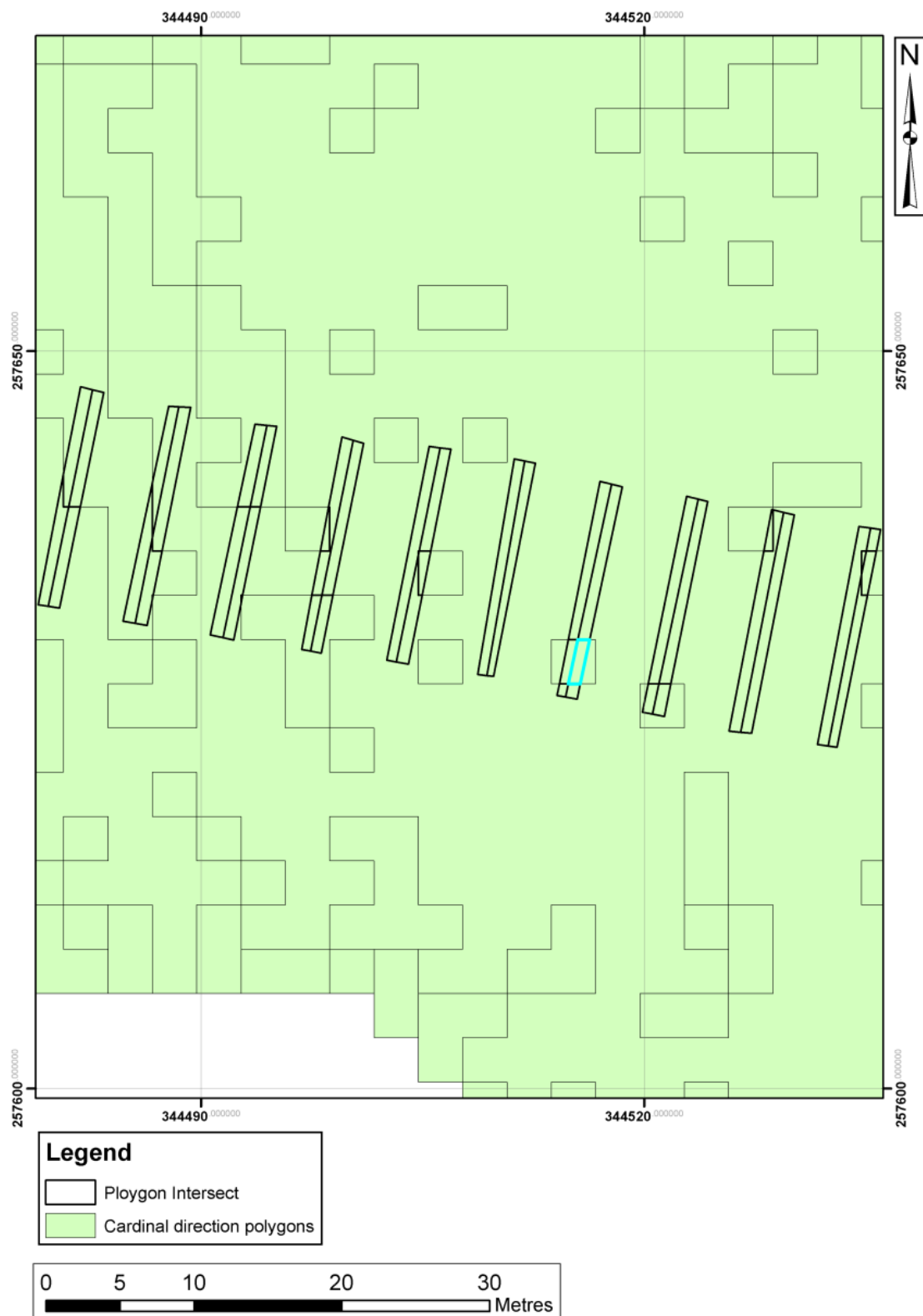


Figure 16 Particular of the intersected polygons, one of the intersections is highlighted in blue.

Table 10 Flow direction classes for each treatment.

Treatment	NW (% ± SE)	N (% ± SE)	NE (% ± SE)
Traditional method	2.9 ± 1.3	73.6 ± 12.6	23.5 ± 13.5
<i>L. multiflorum</i>	2.2 ± 1.0	84.4 ± 7.8	13.4 ± 8.6
<i>L. multiflorum</i> & <i>T. alexandrinum</i>	7.9 ± 3.5	87.9 ± 4.9	4.2 ± 4.2
<i>L. multiflorum</i> & <i>V. villosa</i>	4.2 ± 1.9	62.8 ± 14.5	33.0 ± 16.2

Table 11 Flow direction classes for each area, letters represent homogenous groupings.

Area	NW (% ± SE)	N (% ± SE)	NE (% ± SE)
A	7.8 ± 3.6 ^a	91.4 ± 3.4 ^a	0.8 ± 0.8 ^a
B	3.9 ± 2.2 ^{a,b}	82.1 ± 7.6 ^a	14.0 ± 8.5 ^a
C	0.0 ± 0.0 ^b	53.2 ± 10.7 ^b	46.8 ± 10.7 ^b

2.7 Runoff sampling and runoff analysis

Runoff and sediment were manually collected using 3 x 500 ml sample bottles from each tank after agitating the tanks to re-suspend the deposits and thereby ensure sample homogeneity (for both experiments). Whenever the water level in the tank was very shallow, the runoff was sampled with a scoop. The tanks were emptied after runoff collection to allow repeated measures over time. Water levels in the tanks were measured manually at each sampling event and compared to the level recorded by the sensors.

Several interventions were necessary to perform maintenance on the erosion plots during Experiment 1. The combination of extreme weather events (wind and rain) and soil type caused the tanks to move and become dislodged,

creating gaps where no data were collected. Runoff data were collected twice throughout Experiment 1 and five times throughout the Experiment 2 (Table 12). Runoff was analysed for available N and P, and for total soil load (Clesceri, 1999).

Table 12 Runoff collection periods.

Experiment	Runoff collection period
1	18/12/ 2014 – 13/1/2015
1	22/1/2015– 15/3/2015
2	28/7/2015 – 15/10/2015
2	27/11/2015 – 21/12/2015
2	21/12/2015 -13/01/2016
2	13/1/2016 – 16/2/2016
2	16/2/2016 – 15/3/2016

2.8 Soil sampling and analysis

Soil was sampled (0-15 cm depth) twice using a pallet scoop during each experiment. For Experiment 1 soil was collected on the 5/12/2014 (during the critical period for runoff and erosion) and the 17/03/2015 (before cover crop termination). For Experiment 2, soil samples were collected on the 29/11/2015 and 15/03/2016. Samples comprised of three subsamples taken along the central line of the erosion plots, one from the top, middle and bottom of the slope. During Experiment 2, wheel tracks from the combine harvester impacted the behaviour of the plots, for this reason on the 26/11/2015 soil samples were collected from both within the wheel tracks and outside the wheel tracks in the plot areas. Soil samples for aggregate stability were sampled in November 2015 using a pallet scoop. The samples were placed in sample bags and carefully packed to avoid compaction during transportation. The laboratory

analyses that were performed over the two experiments and the information about when the soil samples were collected are summarised in Table 13.

Table 13 Summary of laboratory analyses and sampling date.

Analysis	Experiment	Sampling date	Reference
pH	1	12/2014 and 3/2015	BS ISO 10390:2005
	2	11/2015 and 3/2016	
Electrical Conductivity	1	12/2014 and 3/2015	BS 7755: Section 3.4:1995
	2	11/2015 and 3/2016	
Total and organic Carbon and total Nitrogen	1	12/2014 and 3/2015	BS 7755 Section 3.8:1995 & BS EN 1354- 2:2001
	2	11/2015 and 3/2016	
Soil organic matter (Loss on ignition)	1	12/2014 and 3/2015	BS EN 13039:2000
	2	11/2015 and 3/2016	
Particle size distribution	1	12/2014	BS 7755 Section 5.4:1998
	2	11/2015	
Bulk density	1	12/2014	Rowell, 1994
Available phosphorous	1	12/2014 and 3//2015	BS 7755: Section 3.6:1995
Available phosphorous	2	11/2015 and 3//2016	BS 7755: Section 3.6:1995
Aggregate stability	2	11/2015	Cooper, 2007

Analysis		Experiment	Sampling date	Reference
Soil microbial biomass (fumigation extraction method)		1	3/2015	BS 7755: Section 4.4.2:1997 & Jenkinson & Powlson, 1976
		2	11/2015 (W. and OW.) and 3/2016	
Phospholipid fatty acid analysis		2	11/2015 (W. [‡] and OW. [†]) and 3/2016	Frostegard et al., 1993
Multiple Substrate Induced Respiration		2	11/2015 (W. [‡] and OW. [†]) and 3/2016	Pawlett et al., 2012
Fungal biomass (Ergosterol)		2	3/2016	Ruzicka et al., 2000
Glomalin related proteins		2	3/2016	Redmile-Gordon et al., 2013
Maize dry yield		2	10/2015	Murphy et al., 1991
Cover crop	Yield	2	3/2016	Murphy et al., 1991
	Plant height stem diameter and stem density	1	3/2015	
	Canopy cover	2	3/2016	

†= Samples collected outside the wheel tracks; ‡=samples collected in the wheel tracks.

2.8.1 Soil chemistry and soil physics analysis

Soil was air-dried and sieved to ≤ 2 mm before analysis for particle size distribution, pH, electrical conductivity (EC), OM, total nitrogen (TN), total organic carbon (TOC) and available phosphorus (AP).

2.8.1.1 Particle size distribution

Particle size distribution followed the British Standard BS 7755 Section 5.4:1998. Samples were treated with hydrogen peroxide to eliminate the OM. The remaining mixture was dispersed with a buffered sodium hexametaphosphate solution and shaken on an end-over-end shaker overnight (18 hours). The samples were dried in an oven set at 105 °C and sieved into a graduated cylinder using a 0.063 mm sieve so that the sand fraction was retained on the sieve. The graduated cylinders were filled with water and placed in a water bath (25 °C) to equilibrate the temperature to 25 °C. The contents of the cylinders were then stirred for 30 s before 25 ml was pipetted out from a depth of 10 cm from the surface of the liquid; the pipetted samples were then dried in an oven at 105 °C. The dried samples contained silt plus clay. After 6 h 23 min another aliquot (25 ml) was pipetted out from a depth of 9 cm from the surface of the liquid and the samples were oven dried. These dried samples contained only clay.

2.8.1.2 Soil pH and electrical conductivity

Soil pH analysis followed the BS ISO 10390:2005. Soil pH was determined using a glass electrode in a 1:5 (volume fraction) suspension of soil in water. Electrical conductivity (EC_{1-5}) analysis followed BS 7755: Section 3.4:1995, 1: 5 soil: water extracts (volume fraction) were shaken for 30-35 minutes on a side-to-side shaker, samples were then centrifuged and filtered. Electrical conductivity₁₋₅ of the filtrate was determined using a Bench Conductivity Meter (Jenway) calibrated with standard solutions prior analysis. EC_{1-5} was used to calculate the saturated electrical conductivity (EC_{sa}):

Equation 1 $EC_{sa} = EC_{1-5} \text{ dS m}^{-1} * \text{conversion factor}$

The conversion factor for a very fine sandy loam is 10.7 (Watling, 2007).

2.8.1.3 Organic matter

Soil organic matter (loss on ignition) analysis followed the BS EN 13039:2000. Samples were placed overnight in an oven set at 105°C to remove the moisture. Subsequently, the mass of the samples was recorded, and the samples were ashed at 450°C in a furnace for 4 h ± 15 min. Loss on ignition is expressed as the mass lost by the ashing.

2.8.1.4 Total organic carbon and nitrogen

Total OC and TN analysis followed BS 7755 Section 3.8:1995 and BS EN 1354-2:22001. Samples were dried in an oven set at 105°C for 2 h ± 10 min and analysed with CHNS 932 Elemental Analyser (LECO Corporation, St. Joseph, MI, USA). Samples were carefully packed into silver-foil boats; samples for total C were treated with hydrochloric acid 4 mol L⁻¹, and placed in the analyser. Carbon contained in the samples was oxidised to carbon dioxide (CO₂) and nitrogen to NO_x and N₂ by heating the soil to 900°C in a flow of oxygen-containing gas that is free from carbon dioxide. A thermal conductivity detector determined the amount of gas produced.

2.8.1.5 Available phosphorus

Available P analysis was based on BS 7755: Section 3.6:1995. Concisely, 5 g of samples were treated with 100 mL of sodium hydrogen carbonate, shaken for 30 minutes and filtered. Sulphuric acid 1.5 mol L⁻¹ (1 mL), ammonium molybdate 0.15% m V⁻¹ (20 mL) and ascorbic acid 1.5% m V⁻¹ (5 mL) were added to 5 mL of filtrate. After 30 minutes of incubation, the samples and working standards were analysed using a spectrophotometer set at 880 nm (Nicolet EvolutionTM 100 UV-Visible Spectrophotometer; Thermo Electron Corporation).

2.8.1.6 Bulk density

Bulk density (mass of soil per unit volume of undisturbed soil) was measured using a bulk density ring (62 cm³). The ring was carefully hammered into the soil using a rubber mallet until the top was at the same level of the soil surface,

using a block of wood to minimise compaction. The weight was determined after drying the samples at 105 °C overnight. Bulk density (ρ_d) was measured using the following equation:

Equation 2 $\rho_d = \frac{m}{V}$

Where:

m =dry weigh (g)

V = volume (cm³)

2.8.1.7 Glomalin-related soil proteins

Glomalin-related soil proteins are a class of molecules related to glomalin that are strongly correlated to aggregate stability (Brady and Weil, 2016); from now on in the document, the term glomalin refers to the entire class of proteins. The methodology to extract and analyse glomalin is based on that of Wright and Upadhyaya (1998), modified by Redmile-Gordon et al., (2013).

Glomalin was extracted by autoclaving 1 g of air-dried sieved soil (≤ 2 mm) for 30 minutes in 8 mL of a 20 mM alkaline citrate solution (pH 7). It was assumed that extraction denatures all proteins with the exception of glomalin. Autoclaved samples were cooled in ice for 10 minutes. Subsequently, samples were centrifuged in a cool rotator at 3500 g for 20 min to pellet the soil particles. It was assumed that glomalin remained in the extract solution.

Glomalin concentration was measured by a modified Lowry assay using bovine serum as standard (Redmile-Gordon et al., 2013). The Lowry assay consists of two phases The Biuret reaction and the Folin–Ciocalteu reaction. During the Biuret reaction, copper sulphate binds to proteins, forming a peptide complex and reducing Cu^{2+} to Cu^{1+} . Subsequently, Folin-Phenol is reduced by Cu^{1+} , forming a soluble blue complex that can be detected at 750 nm. A calibration curve was created using bovine serum albumin (BSA) dissolved in phosphate buffered saline (PBS; 0, 15, 30, 60 $\mu\text{g mL}^{-1}$), samples were diluted 1:2 folds with PBS to ensure protein concentration was in range with the calibration curve. Two dye reagents were prepared. Reagent A was formed by 0.14M of

copper sulphate, 0.66M of sodium potassium tartrate and 0.35N of sodium hydroxide combined proportions of 1:1:100 (v:v:v). Reagent B was prepared in the same way, except the copper sulphate solution was excluded and volume substituted with deionised water. Two sets of plate readers were prepared labelled A and B. Twelve replicated samples and standards were placed in each well of plate A (50 μ L). Half of the unknown sample was spiked with 50 μ g mL⁻¹ BSA. The same was repeated for plate B. Subsequently, 100 μ L of reagent A was added to each well of the microplates A, and 100 μ L of reagent B was added to each well of the microplates B. Then 100 μ L of Folin-Phenol reagent diluted tenfold in distilled water was added to both plates. The plates were incubated for 30 minutes at room temperature in the dark and read with a plate reader set at 750 nm for 150 ms per well.

2.8.1.8 Aggregate stability

Aggregate stability was assessed using a gravity fed rainfall tower to simulate a natural rainfall event (Cooper, 2007). The soil was broken up by hand into aggregates and left to air dry in trays. Once dried, the aggregates were separated into eight size classes: > 12.5 mm, 9.5 mm - 12.5 mm, 9.5 mm – 5 mm, 4 mm – 5 mm, 3.35 mm – 4 mm, 2 mm - 3.35 mm, 1 mm - 2mm and <1mm using a stack of sieves. The soil was placed in the sieve at the top of the stack. The stake was shaken ten times and hit three times; the action was repeated three times. The size fraction 4mm-5mm was chosen for the stability test since Cooper (2007) found that there were no significant differences in stability between the different classes. Additionally, it was easier to saturate aggregates of that size compared to smaller aggregates and to move them compared to larger aggregates.

For each replication, 15 g of dry aggregates per treatment were saturated with tap water. Aggregates were placed on top of a voile of multiple layers of moist paper for a minimum of 1 h (Nimmo and Perkins, 2002). Once saturated the aggregates were carefully moved into a 1 mm mesh sieve with a receiver for the analysis.

The rain tower used was 9 m high; this ensures that the raindrops reach terminal velocity (Low, 1954). The artificial rain drops were produced by 860 hypodermic needles, gauge size 24 (0.559 mm) (Jensen Global 2006), placed at the bottom of a tray filled with water. The head of the water above the needles was kept constant by a constant flow and a weir. Raindrop size was randomised by a mesh placed 1m below the tray.

Rainfall intensity was measured using a laser disdrometer (Present Weather Sensor Parsivel; Ott Messtechnik GmbH & Co. KG). The intensity was calibrated by modifying the head of the water in the tray and the height of the weir. Raindrop size distribution was measured using catch cups uniformly distributed under the tower. During the test two rainfall intensities were used: $86.8 \text{ mm h}^{-1} \pm 1.1 \text{ SD}$ and $47.7 \text{ mm h}^{-1} \pm 1.2 \text{ SD}$, corresponding to 1-50 years storm and 1-10 year return storms respectively for the river Lugg catchment (Cooke, personal communication). The rainfall characteristics were measured and are summarised in Table 12. The test was performed using four sieves per time. After raining on the samples the soil and the water contained in the receiver were placed into drying tins and left to air dry. The tins were weighed to calculate the percentage of stable aggregates (retained by the sieve).

Table 14 Artificial rain specifications

RI[†] Cup method	RI[†] Laser disd.[‡]	Rate of KE[§] expenditure	KE[§] content	D50 ± SD	N. of rep.	Time
± SD	± SD	± SD	± SD			
(mm h⁻¹)	(mm h⁻¹)	(J m⁻² h⁻¹)	(J m⁻² mm⁻¹)			(min)
86.8 ± 1.1	83.2 ± 2.9	1015.9 ± 114.6	12.2 ± 1.0	1.4 ± 0.1	4	15
47.7 ± 1.2	50.7 ± 7.5	895.6 ± 187.8	17.5 ± 1.2	2.2 ± 0.4	4	15

† = Rainfall Intensity, §= Kinetic Energy; ‡= disdrometer

2.8.2 Soil microbiological analysis

2.8.2.1 Microbial Biomass

Soil was kept in the fridge (5 °C) until the day of the analysis and sieved fresh (≤ 2 mm). Soil microbial biomass analysis (fumigation-extraction method) was based on BS 7755: Section 4.4.2:1997, which is based on Jenkinson & Powlson, (1976). Samples were divided into two subsets, one of which was treated with fumigation. Fresh samples, containing a mass equivalent to 12.5 g of oven-dry sample, were fumigated with chloroform for 24 h in a desiccator to lyse living microbial cells. The chloroform was eliminated by repeated evacuation (six times, 2 min each). A solution of potassium sulphate (0.5 mol L^{-1}) was added to both non-fumigated and fumigated samples, which were then shaken for 30 min and filtered to extract the organic carbon. Sodium polyphosphate is added to the extract (5 ml) to dissolve precipitates of calcium sulphate.

The OC content of the extract was determined using a SFA-2000 segmented flow analyser (Burkard Scientific, Uxbridge, UK). Samples were acidified and saturated with air not containing carbon dioxide; this process eliminated the inorganic C in the samples. Subsequently, OC was converted to CO_2 by the addition of acidified potassium sulphate and by exposition to UV light. The CO_2 released changes the colour of a buffered phenolphthalein solution that can be detected at 550 nm. The MBC was calculated using Equations 3, 4 and 5. The extractable C from fumigated and non-fumigated samples was calculated using Equation 3.

Equation 3 Extractable C ($\mu\text{g g}^{-1}$ dry sample) = $[(S \times D) - (B \times D_B)] \times ((P_K + S_W - D_W) / D_W)$

$S = C \text{ (mg L}^{-1}\text{) of the sodium polyphosphate treated sample extract}$

$B = C \text{ (mg L}^{-1}\text{) of the sodium polyphosphate treated blank}$

$D = \text{dilution factor of the sample with sodium polyphosphate, which equals to 2}$

$D_B = \text{dilution factor of the blank with sodium polyphosphate, which equals to 2}$

$P_K = \text{volume of potassium sulphate (50 mL) used to extract the soil samples}$

S_W = mass (g) of field-moist sample

D_W = dry mass (g) of sample used (Equation 3)

Equation 4 $D_W = (S_W \times 100) / (100 + W_{H_2O})$

D_W = dry mass (g) of the sample

S_W = mass (g) of field-moist sample

W_{H_2O} = water content (%) on an oven-dry basis

Once the extractable C is calculated, the MBC is calculated as the difference between C extracted from fumigated samples, and C extracted from non-fumigated samples, divided a conversion factor (Equation 4).

Equation 5 $B_C (\mu\text{g} / \text{g dry sample}) = E_C / K_{EC}$

B_C = Microbial Biomass Carbon

E_C = (mean mass of organic C extracted from fumigated samples) – (mean mass of organic C extracted from non-fumigated samples)

K_{EC} = 0.45 (Joergensen, 1996)

2.8.2.2 Fungal biomass

Fungal biomass was measured using ergosterol as a proxy (Ruzicka et al., 2000). Soil samples (4 – 6 g) were divided into two sets. One set of samples was spiked with a solution of ergosterol (100 μg) in n-hexane: 2-propanol (1mL, 98:2 v/v). All samples were left at room temperature for 15 min, before 10mL of methanol: ethanol (4:1 v/v) was added. Samples were stored at 4°C for 2 h, and then added 20mL of n-hexane: 2-propanol solvent (98:2 v/v; 19 ml for the spiked samples). Ergosterol was extracted by sonicating the samples in an ice bath using a sonic probe at 150 W for 2 min. The top layer was centrifuged at 7000 g for 10 min. Ergosterol content was determined by HPLC set at 282 nm (Kontron 525 pump, 565 auto-sampler, 535 UV detector, Knauer degasser, EZChrom Elite software; SCI Tek instruments, Olney, UK), with a column 150 x 4.6 mm Lichrosorb Si 60 (10 μm particle size; Phenomenex, Macclesfield, UK).

2.8.2.3 Phospholipid fatty acid analysis

Phospholipid fatty acid analysis was based on the method developed by Frostegård et al., (1993). Soil samples were freeze-dried before the analysis. Lipids were extracted from soil samples (10 g) using 20 mL Bligh and Dyer solvent (citrate buffer, chloroform, methanol, 0.8:1:2 v/v) (Bligh and Dyer, 1959) with an addition of 0.005% w/v butylated hydroxyl-toluene as an anti-oxidant. Samples were sonicated for 30 min in an ultrasonic bath and subsequently centrifuged at 700 RCF for 10 min. The upper layer (organic fraction) was retained, and 4 ml of chloroform and 4 ml citrate buffer were added. Samples were centrifuged at 700 RCF for 10 min, and the top aqueous layer was discharged using a vacuum pump. The organic less layer was then dried under N₂ in an evaporator set at 36°C; samples were kept frozen ($\leq 20^{\circ}\text{C}$) until required for fractionation phase.

In the fractionation phase, the lipids are separated into neutral lipids, glycolipids and polar lipids using solid phase extraction (SPE) columns (3 ml/ 500 mg silica Sep-Pak Vac™, Waters Chromatography, Milford MA, USA). SPE cartridges were placed onto a vacuum manifold, 0.5 g sodium sulphate was added to absorb any water, and the cartridges were washed with 2 x 2 ml chloroform. The dry lipid extracts were defrosted at room temperature, re-suspended with 1 ml chloroform, transferred in the SPE cartridges and washed with 1 ml of chloroform onto the cartridge using gravity only. Chloroform (5 ml) eluted the neutral lipids onto the cartridge under vacuum and acetone (12 ml) eluted the glycol lipids; these lipids were discarded. Finally, the polar lipids (phospholipids) were eluted with 8 ml of methanol in a clean glass bottle, dried under N₂ in an evaporator set at 36 °C and frozen (- 20 °C) until the next phase (Mild Alkaline Methanolysis).

The Mild Alkaline Methanolysis phase transforms the phospholipids in fatty acid methyl esters (FAMES). Samples were reconstituted with 1 ml of 1:1 toluene: methanol. The phospholipids were then converted to FAMES following the addition of 1 ml of 0.2 M methanolic potassium hydroxide, which hydrolyses the lipids; the samples were left to react for 30 min at 25°C. The reaction was

stopped by adding 0.25 ml of 1 M acetic acid. 5 ml of 4: 1 v/v Hexane: Chloroform (and 3 ml of deionised water were added to the samples, which were then centrifuged to separate the two phases (organic and aqueous). The organic phase was collected using a Gilson pipettor and then dispensed through a 10 ml pipette tip packed with glass wool and sodium sulphate into clean glass bottles, rinsed with a few ml of hexane, and dried under N₂ in an evaporator set at 25 °C. The dried FAMES were reconstituted with 200 µL of hexane for gas chromatography (GC; 6890N, Agilent Technologies, Stockport, UK).

The GC was equipped with a flame ionization detector (GC-FID), split/splitless injector and an HP-5 (Agilent Technologies) capillary column (30 m length, 0.32 mm ID, 0.25 µm film). Helium was used as the carrier gas (1 ml min⁻¹). FAMES were separated by using a temperature program that started at 50°C for 1 min (splitless hold time). Subsequently, the program increased the temperature by 25°C per min until reaching 160°C, followed by an increase of 2°C per min until 240°C, and 25°C per min until 310°C. Samples were injected (1 µL) using an autosampler (injector temperature of 310°C), and FAMES detected using an FID operating at 320°C.

Standard mixtures of known PLFAs (SUPELCO) were used to identify the primary PLFAs in the soil samples and determine, by mass comparison, the relative concentrations of each PLFA. The FAMES selected for the analysis were 15:0, 15:0i, 15:0ai, 16:0i, 16:1Ω5, 17:0i, 17:0ai, 17:0c, cyc17:0 isomer, 17:1Ω7, 18:2Ω6, 9 and 19:0c. The FAME 18:2Ω6, 9 was used as the indicator of the incidence of saprotrophic fungi and ectomycorrhizae. 16:1Ω5 was used as the indicator of AMF. The sum of the mol% of the fatty acids 15:0i, 15:0ai, 16:0i, 17:0i, 17:0ai, 17:0c, 19:0c as the indicator of Gram-positive bacteria and 16:1Ω7c, 16:1Ω8 as the indicator of Gram-negative bacteria. Gram-positive and Gram-negative bacteria plus 15:0, cyc17:0isomer and 17:1Ω7 were used as indicators of total bacteria. Fungi/Bacteria was calculated using total bacterial markers over 18:2Ω6,9 (Frostegard, 1996; Olsson, 1999; Pawlett et al., 2012).

2.8.2.4 Multiple substrates induced respiration and metabolic quotient

Multiple substrates induced respiration (MSIR) was assessed using a multi-channelled automated conductimetric respirometer, the Rapid Automated Bacterial Impedance Technique (RABIT; Don Whitley, Shipley, UK) (Pawlett et al., 2012). This technique was used to describe the functional profile of the microbial community using the catabolic responses of the community after the addition of readily degradable C substrates (Degens and Harris, 1997).

The soil was incubated for one week in the dark at a constant temperature (25°C). Samples were kept at a friable state by adding water or allowing evaporation. The moisture content of the samples was measured before the analysis. 0.5 ml of the following substrates were added to 1 g of sample: D-(+)-glucose (75mM), L- arginine (15mM), L-glutamine (15mM), citric acid (100mM), alpha-Ketoglutaric acid (100mM), and deionised water for basal respiration measurement.

Respiration rates were calculated using respiration values after 240 minutes and after 360 minutes from the start of the analysis (Ritz et al., 2006), because data before the 240th minute is often noisy (Pawlett, 2016, personal communication). The rates were reported in $\mu\text{g of CO}_2 \text{ g}^{-1}$ per dry weight of soil. The metabolic quotient ($q\text{CO}_2$) was calculated as the ratio between soil basal respiration rate and its MB (Equation 6) (Anderson and Domsch, 1993).

$$\text{Equation 6 } q\text{CO}_2 = \frac{\text{Soil Basal Respiration rate } \mu\text{g (CO}_2 \text{ g}^{-1} \text{ soil h}^{-1})}{\text{Microbial biomass (mgC g}^{-1} \text{ soil)}}$$

2.9 Maize and cover crop assessment

2.9.1 Dry yield

Maize and cover crop yield were estimated using the crop cut method (Murphy et al., 1991), using a 0.25 m² quadrat. For maize, each plot was divided into 15 subunits, three units per plot were selected, the number of maize plants was

counted, and two plants (top and bottom plant in the unit) were selected for biomass assessment. Plants were dried at 60°C for three days and weighed for dry weight estimates. For cover crops yield assessment, the same methodology was followed, but all above-ground biomass inside the quadrat was sampled. Maize and cover crop dry yield were only assessed for Experiment 2 since Experiment 1 was established after the harvest.

2.9.2 Cover crop characterisation

During Experiment 1 stem density, stem diameter and crop height were measured using a 1 m² quadrat separated into 16 smaller quadrats (25 cm²). The allocation of the quadrat was randomly selected by dividing the plot into 15 subunits. The quadrat was placed in the centre of the unit and three randomly selected smaller quadrats (25 cm²) were used to count stem density, stem diameter and crop height.

2.9.3 Estimation of canopy cover

Cover crop cover was estimated using a digital imagery technique during Experiment 2 (Waine et al., 2014). One photo per plot, from 1 m height was taken using a quadrat (0.25 m²) (Figure 17), especially designed with four colourful targets in the corners. Location of the quadrat was randomly selected by divided the plot into 30 subunits. Small circles were superimposed on cropped and rectified images (Python; Figure 18) and their interception with the image of the cover crop was used to quantify the canopy cover (Simms, 2016; Appendix D). If the majority of the circle was filled by a cover crop image the circle was considered cover crop, otherwise it was considered bare ground. The percentage of canopy cover was calculated as the percentage of the cover crops circles over the total number of circles imposed on the image.



Figure 17 Example of a picture before the edits.



Figure 18 Example of a rectified picture with superimposed circular dots.

2.10 Statistical analysis

Statistical analysis was performed using the software STATISTICA (Statsoft, Inc. 2010). The following tests were conducted during the two experiments (Tables 15 and 16):

- Box-Cox transformation was used to normalise the data if logging and square rooting was not adequate. Variables ≤ 0 were shifted to 1.
- Repeated measures ANOVA was used to find homogenous groups between the treatments during Experiment 1 and Experiment 2 using Time as within-subjects factor. Significant differences between locations were checked for Experiment 2. Tests automatically checked for sphericity and rejected the results if the assumption was not met. Additionally, data were checked for bivariate-normality looking at the distribution of the quantile-quantile and probability-probability plots (Burdenski, 2000); distributions close to normality were also accepted and outliers were excluded when necessary. Significant difference was set at $P < 0.05$.
- One-way ANOVA followed by post-hoc Fisher LSD comparison was performed to find homogenous groups between the treatments during Experiment 1 and Experiment 2 when the measure was not repeated in time. Significant differences between locations and presence or absence of wheel tracks were checked for Experiment 2. Data was checked for normality and homogeneity (Levene's test); if test assumptions were not met data was logged, square rooted or transformed using Box-Cox. Significant difference was set at $P < 0.05$.
- Principal component analysis (PCA) was used to analyse the relative percentage of the fatty acids of the PLFA and the multiple respiratory rates of the MSIR. The resultant factors scores were analysed for significant differences using One-way ANOVA between treatments.

Table 15 Summary of the statistical analyses and normalisation of data collected during Experiment 1.

Analysis	Sampling date	Type of normalisation	Statistical test
pH	12/2014	Not required	Repeated measures ANOVA
	3/2015		
Electrical Conductivity	12/2014	Not required	Repeated measures ANOVA
	3/2015		
Organic Carbon and Total Nitrogen	12/2014	Not required	Repeated measures ANOVA
	3/2015		
Organic matter	12/2014	Not required	Repeated measures ANOVA
	3/2015		
Particle size distribution	12/2014	Box-Cox for Sand and Clay; Log ₁₀ for Silt	ANOVA
Bulk density	12/2014	Not required	One-way ANOVA
Microbial biomass	3/2015	Not required	One-way ANOVA
Available phosphorus	12/2014	Not required	Repeated measures ANOVA
	3/2015		
Runoff	18/12/2014 - 13/01/2015	Box-Cox	One-way ANOVA
Analysis	Sampling date	Type of normalisation	Statistical test

Analysis	Sampling date	Type of normalisation	Statistical test
Runoff	22/01/2015 - 15/03/2015	Box-Cox	One-way ANOVA
	Cumulative values	Box-Cox	One-way ANOVA
Nutrients in the runoff	22/01/2015 - 15/03/2015	Box-Cox	One-way ANOVA
Soil loss	22/01/2015 - 15/03/2015	Box-Cox	One-way ANOVA
Cover crops Plant height (cm), stem diameter (cm) and stem density (m ⁻²)	3/2015	Not required	One-way ANOVA

Table 16 Summary of the statistical analyses and normalisation of data collected during Experiment 2.

Analysis	Sampling date	Type of normalisation	Statistical test
pH	11/2015	Not required	Repeated measures ANOVA
	3/2016		
Electrical Conductivity	11/2015	Not required	Repeated measures ANOVA
	3/2016		

Analysis	Sampling date	Type of normalisation	Statistical test
Organic Carbon	11/2015	Not required	Repeated measures ANOVA
	3/2016		
Analysis	Sampling date	Type of normalisation	Statistical test
Total Nitrogen	11/2015	Not required	Repeated measures ANOVA
	3/2016		
Organic matter	11/2015	Not required	Repeated measures ANOVA
	3/2016		
Available phosphorus	11/2015	Not required	Repeated measures ANOVA
	3/2016		
Particle size distribution	11/2015	Box-Cox for Sand and Clay	MANOVA
Aggregate stability	3/2016	Not required	One-way ANOVA
Microbial biomass	11/2015	Not required	One-way ANOVA
	W versus OW		Repeated measures ANOVA
	11/2015		
	3/2016		

Analysis	Sampling date	Type of normalisation	Statistical test
Phospholipids fatty acid analysis	11/2015 W versus OW	Not required	One-way ANOVA on Principal components
	11/2015	Not required	Repeated measures ANOVA on Principal components
	3/2016		
Analysis	Sampling date	Type of normalisation	Statistical test
Fatty acids indicators (Total bacteria, fungi, F/B)	11/2015 W versus OW	Not required	One-way ANOVA
	11/2015	Not required	Repeated measures ANOVA
	3/2016		

Analysis	Sampling date	Type of normalisation	Statistical test
Multiple Substrate Induced Respiration	11/2015 W versus OW	Not required	One-way ANOVA on Principal components
	11/2015	Not required	Repeated measures ANOVA on Principal components
	3/2016		
Fungal biomass	3/2016	Square root	One-way ANOVA
Glomalin related proteins	3/2016	Not required	One-way ANOVA
Runoff	28/07/2015 – 15/10/2015	Box-Cox	One-way ANOVA
	27/11/2015 - 21/12/2015		
	21/12/ 2015 - 13/01/2016		
	13/01/2016 – 16/02/2016		

Analysis	Sampling date	Type of normalisation	Statistical test
Runoff	16/02/2016 – 15/03/2016	Box-Cox	One-way ANOVA
	Cumulative runoff	Box-Cox	One-way ANOVA
Soil loss	28/07/2015 – 15/10/2015	Box-Cox	One-way ANOVA
	27/11/2015 - 21/12/2015		
	21/12/ 2015 - 13/01/2016		
	13/01/2016 – 16/02/2016		
	16/02/2016 – 15/03/2016		
	Cumulative soil loss	Box-Cox	One-way ANOVA

OW= Samples collected outside the wheel tracks; W=samples collected in the wheel tracks.

3 RESULTS

3.1 Survey Results

The survey was sent to the members of the Maize Growers Association; not all the growers participating in the survey answered all the questions, and the average number of responses per question was low (15). More than half of the respondents reported that they encountered runoff and soil erosion (Figure 19), of these growers 95% was dealing with the problem by implementing soil erosion control measures.

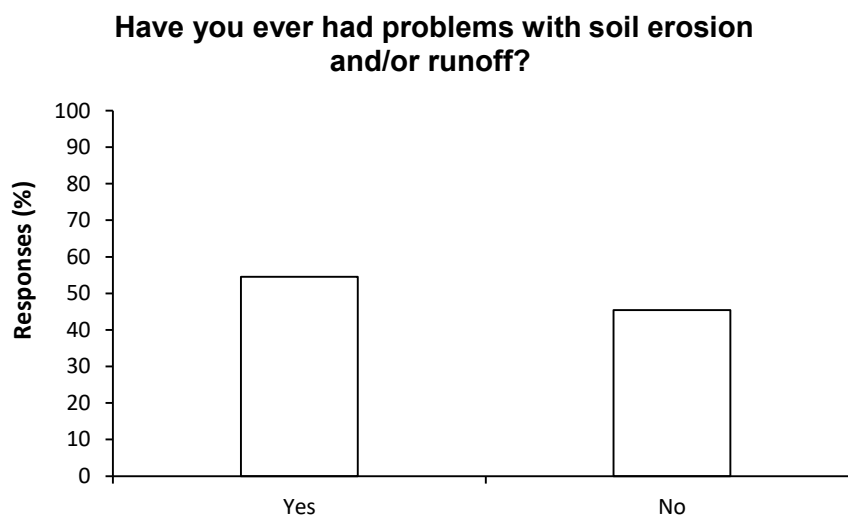


Figure 19 Comparison of responses to the dichotomous question: have you ever had problems with soil erosion and/or runoff? (Yes n=30, No n=25).

Cover crops were the preferred management option to control runoff and erosion among the growers who experienced runoff and soil erosion, followed by buffer strips and changes to the ploughing regime (Figure 20). Many growers also indicated that they implemented other options, such as planting winter wheat (*Triticum aestivum*) and ploughing cross slope post-harvest.

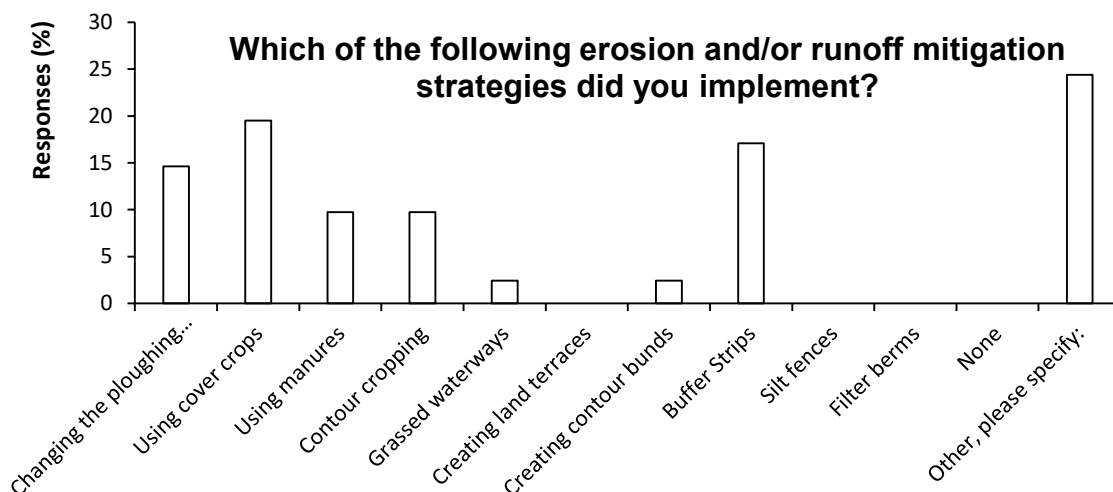


Figure 20 Comparison of responses to the multiple choices question: which of the following erosion and/or runoff mitigation strategies did you implement? (n=20).

Maize growers were asked if they had or would consider sowing cover crops (Figure 21). The majority (80%) of growers who encountered runoff and soil erosion considered implementing cover cropping, while only 60% of growers that did not experience runoff and erosion would consider it.

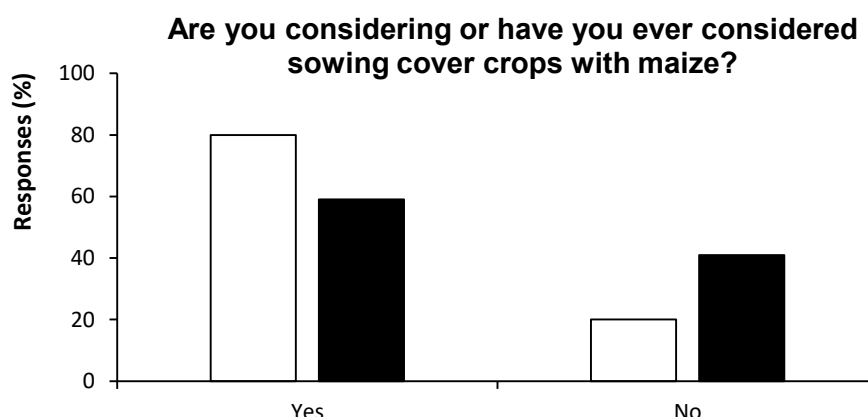


Figure 21 Comparison of responses to the dichotomous question: are you considering or have you ever considered sowing cover crops with maize? Growers who experienced erosion (white), n=20. Growers who did not experience erosion (black), n=22.

Growers were asked to identify one or more reasons why they used cover crops (Figure 22). The principal reason to sow cover crops among the growers who experienced runoff and soil erosion was to decrease soil erosion (88%), followed by improve soil structure (81%) and increase soil organic matter (63%). On the contrary, the main reasons Considering growers who did not encounter runoff and soil erosion were to increase organic matter (62%) and improve soil structure (62%; Figure 22).

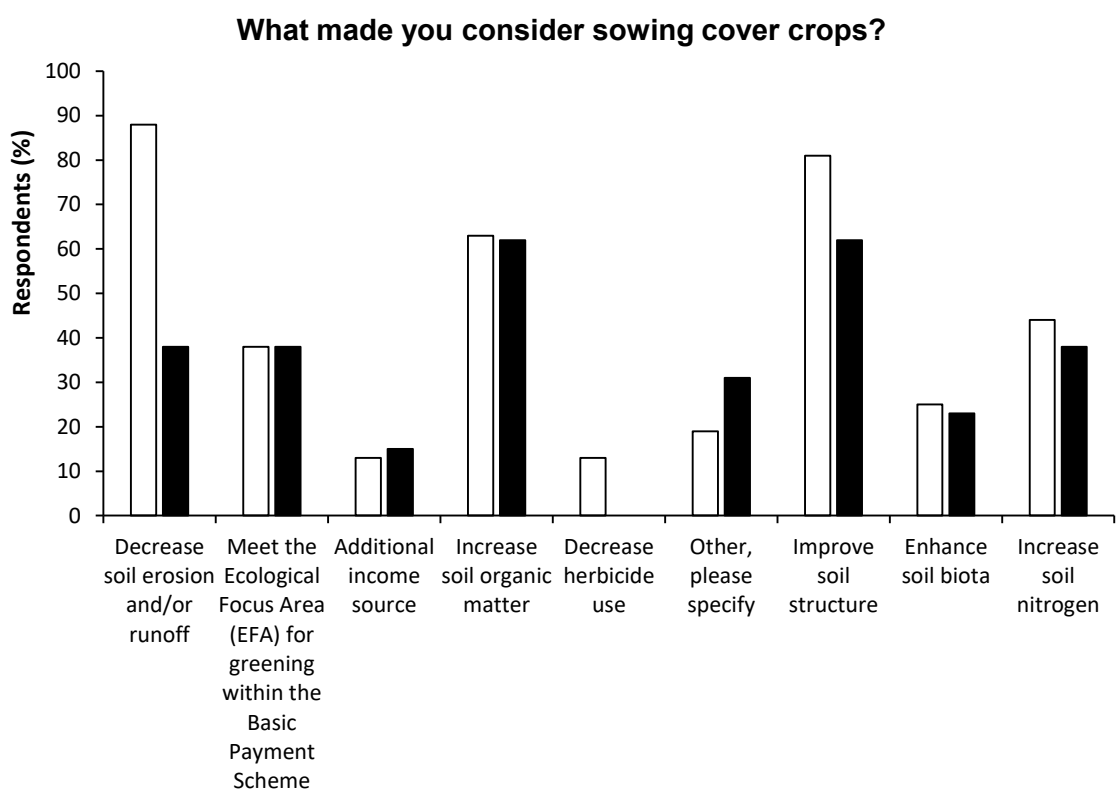


Figure 22 Comparison of responses to the multiple choices question: what made you consider sowing cover crops? Growers who experienced erosion (white), n=16. Growers who did not experience erosion (black), n=13.

Growers were asked the prime motivation to not grow cover crops. The response to this question was low (14 responses; Figure 23). The 25% of the growers who experienced runoff and erosion were afraid of cover crops

competition with maize; while the majority of the respondents who did not experience runoff and erosion had other reasons not to sow cover crops such as growing wheat (*Triticum* sp.). In reference to the maize growers who did not consider planting cover crops 78% of the respondents did not see advantages in the practice, 22% were afraid cover crops would have an adverse impact on yield and 22% had other reasons such as they grow grain maize, which leaves more residues on the surface (lower erosion risk), or they do not have sloping fields.

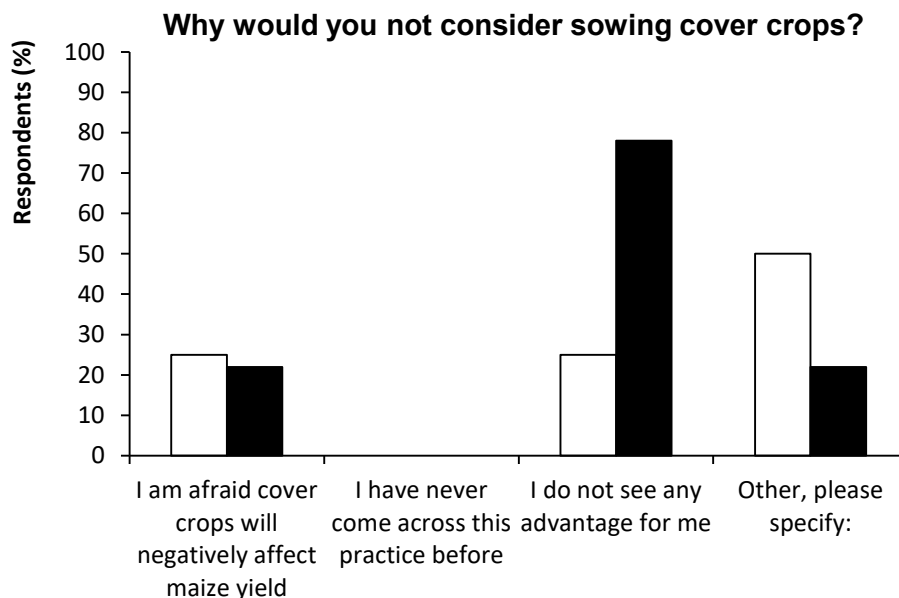


Figure 23 Comparison of responses to the multiple choices question: why would you not consider sowing cover crops? Growers who experienced erosion (white), n= 4. Growers who did not experience erosion (black), n=10.

Participants were asked to appoint their preferences on a list of cover crops. Only two cover crops had more positive responses than negative among the growers who encountered runoff and soil erosion (Figure 24). The two species were ryegrass (*Lolium* sp.) that was recommended by 74%, and forage rye (S.

cereale) approved by 56%. The 60% suggested using other species, such as winter wheat (*Triticum aestivum*). Vetch (*Vicia* sp.) and clover (*Trifolium* sp.) were recommended by 30% of the respondents.

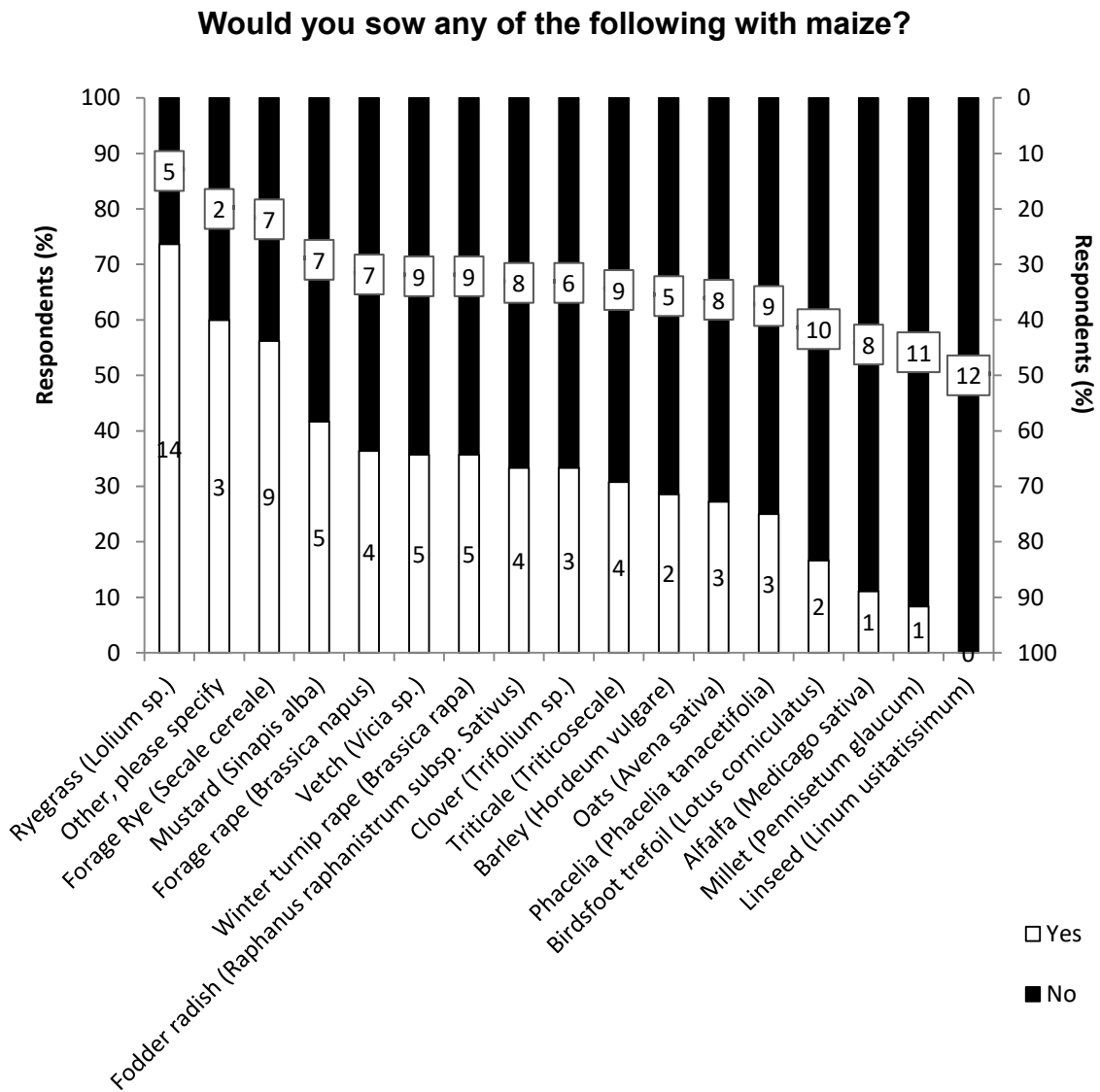


Figure 24 Comparison of responses to the multiple choices question: would you sow any of the following with maize? Growers who experienced erosion problems. n values are shown across the histograms.

Three cover crop species had more positive responses than negative among the growers who did not experience runoff and soil erosion, (Figure 25). Those crop species were clover (*Trifolium* sp.) that was recommended by 86%, ryegrass (*Lolium* sp.; 62%) and forage rye (*Secale cereale*; 60%). Additionally, vetch (*Vicia* sp.) and forage rape (*Brassica napus*) had a positive response from 50% of the respondents.

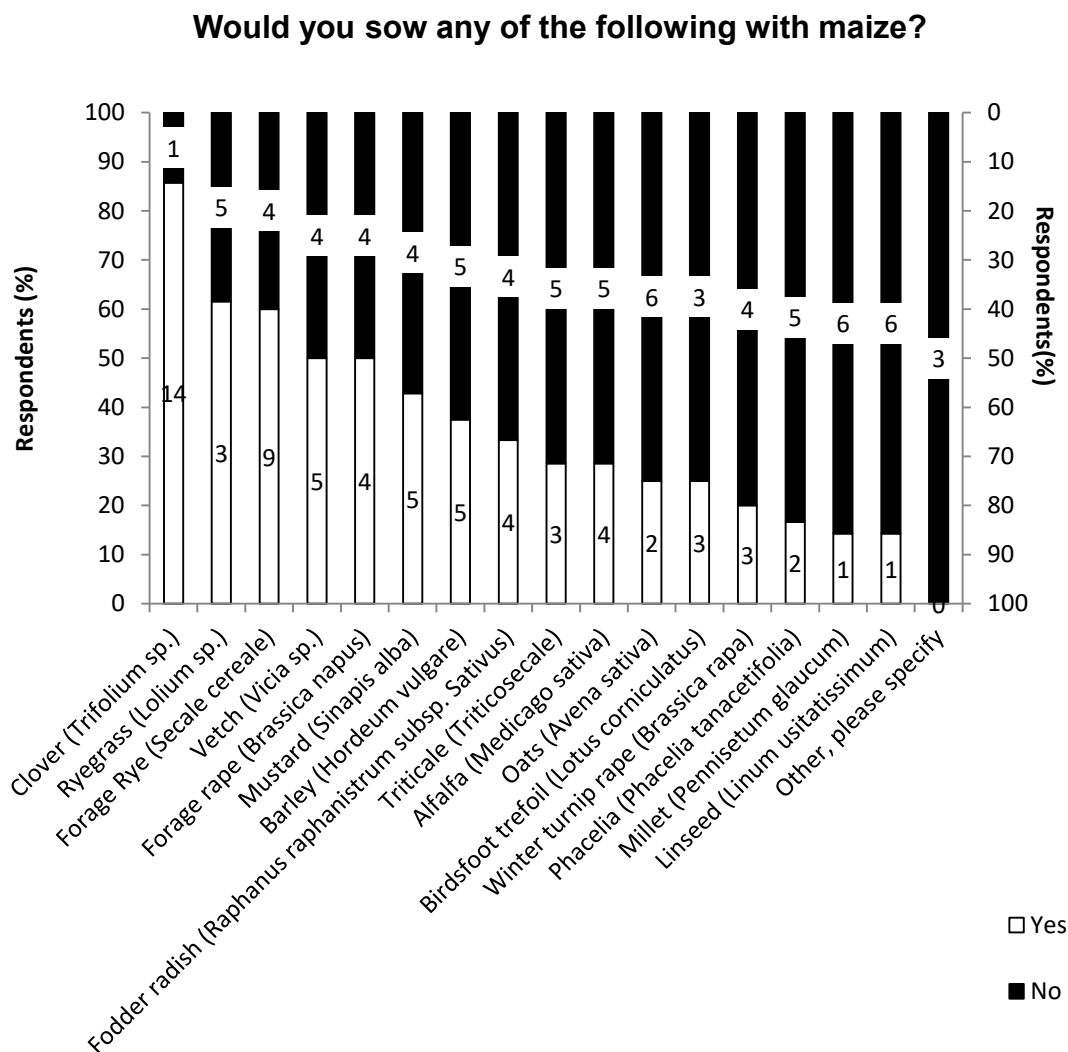


Figure 25 Comparison of responses to the question: would you sow any of the following with maize? Growers who did not experience erosion problems. n values are shown across the histograms.

Growers were also asked to justify their choice of cover crop. Respondents that experienced runoff and erosion would grow forage rape (*Brassica napus*), forage rye (*Secale cereale*) and ryegrass (*Lolium* sp.) primarily for their economic value (Figures 26, 27, 28 and 29). They would plant barley (*Hordeum vulgare*) because of the economic value of the crop. However, those who would not want to plant barley were concerned about the competition of the crop with maize. Similarly, growers would not plant alfalfa (*Medicago sativa*) and millet (*Pennisetum glaucum*) predominantly because of the possible competition with maize. Clover (*Trifolium* sp.), birdsfoot trefoil (*Lotus corniculatus*), fodder radish (*Raphanus raphanistrum* subsp. *Sativus*), mustard (*Sinapis alba*), phacelia (*Phacelia tanacetifolia*) and vetch (*Vicia* sp.) would be chosen mostly as green manures. Linseed (*Linum usitatissimum*) and clover (*Trifolium* sp.) were not chosen because considered not suitable for the area.

Growers who did not experience runoff and soil erosion would plant clover (*Trifolium* sp.), fodder radish (*Raphanus raphanistrum* subsp. *Sativus*), winter turnip rape (*Brassica rapa*) and vetch (*Vicia* sp.) as green manures. Winter turnip rape (*Brassica rapa*), oats (*Avena sativa*), ryegrass (*Lolium* sp.), barley (*Hordeum vulgare*) and alfalfa (*Medicago sativa*) were chosen for their economic value. Forage rape (*Brassica rapa*), barley (*Hordeum vulgare*) and phacelia (*Phacelia tanacetifolia*) were rejected because of the potential competition with maize. None of the cover crops was excluded because considered not suitable for the area.

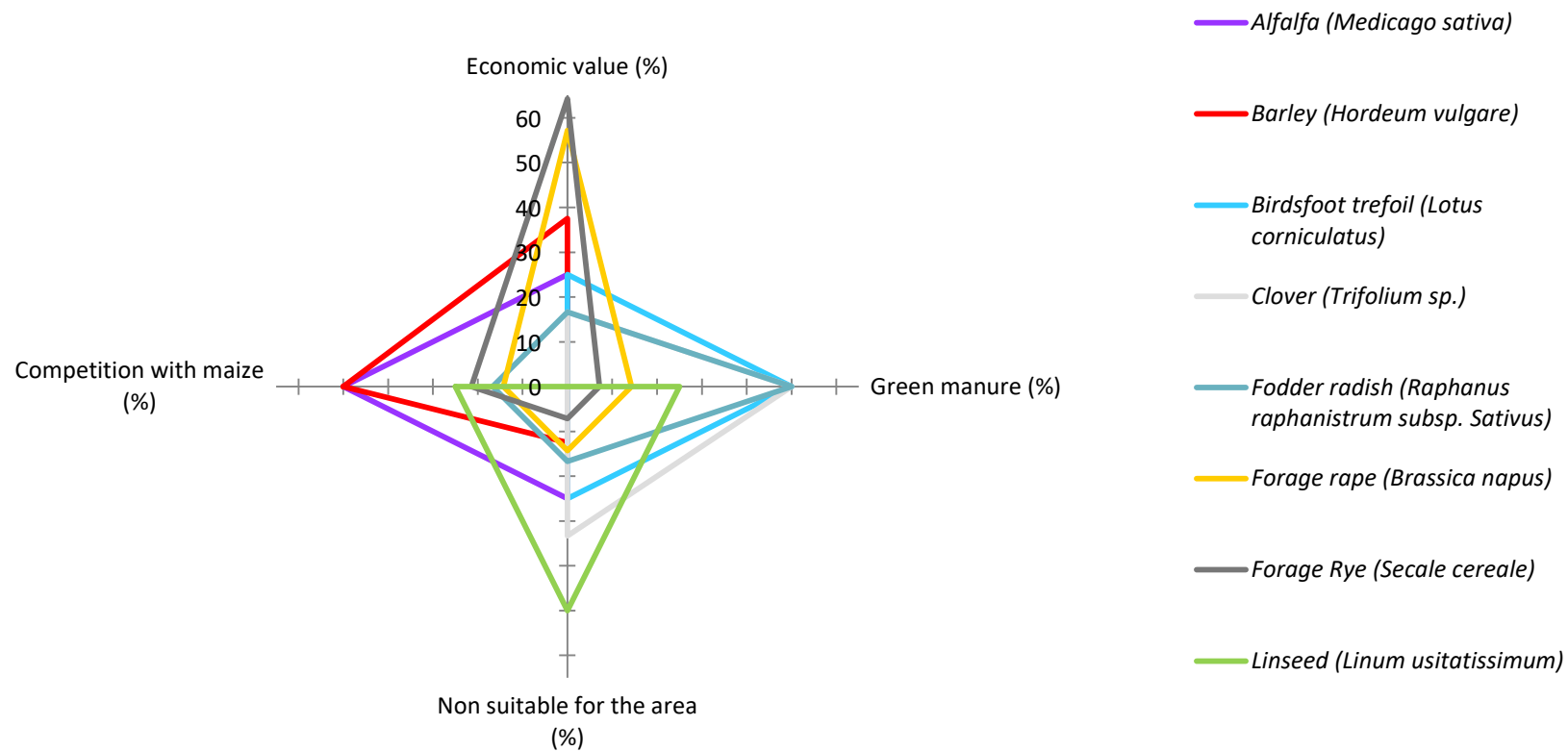


Figure 26 Comparison of responses to the question: why you sow/not sow any of the following with maize? (Growers who experienced erosion problems range = 4-14).

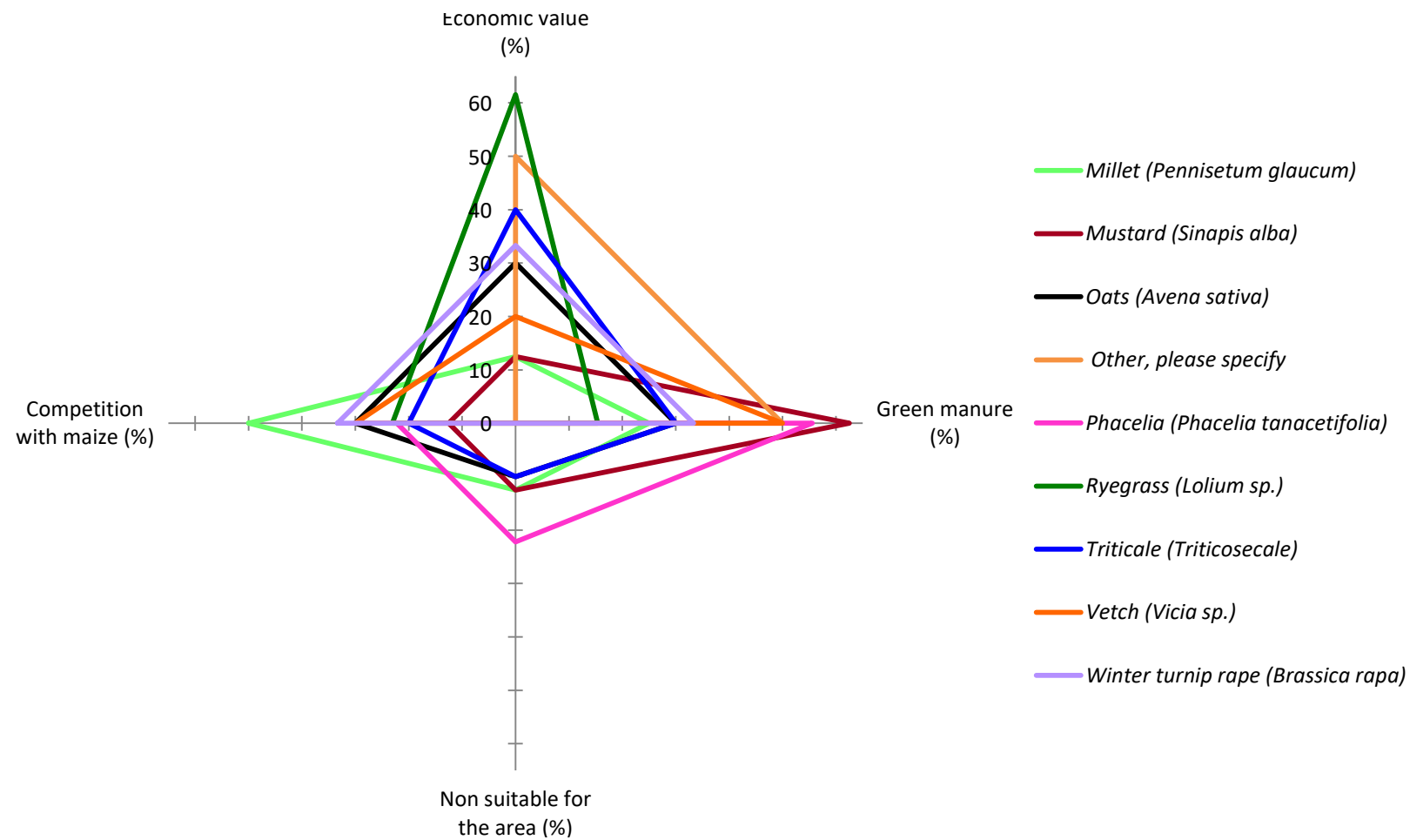


Figure 27 Comparison of responses to the question: why you sow/not sow any of the following with maize? For growers who experienced erosion problems (n range = 4-14).

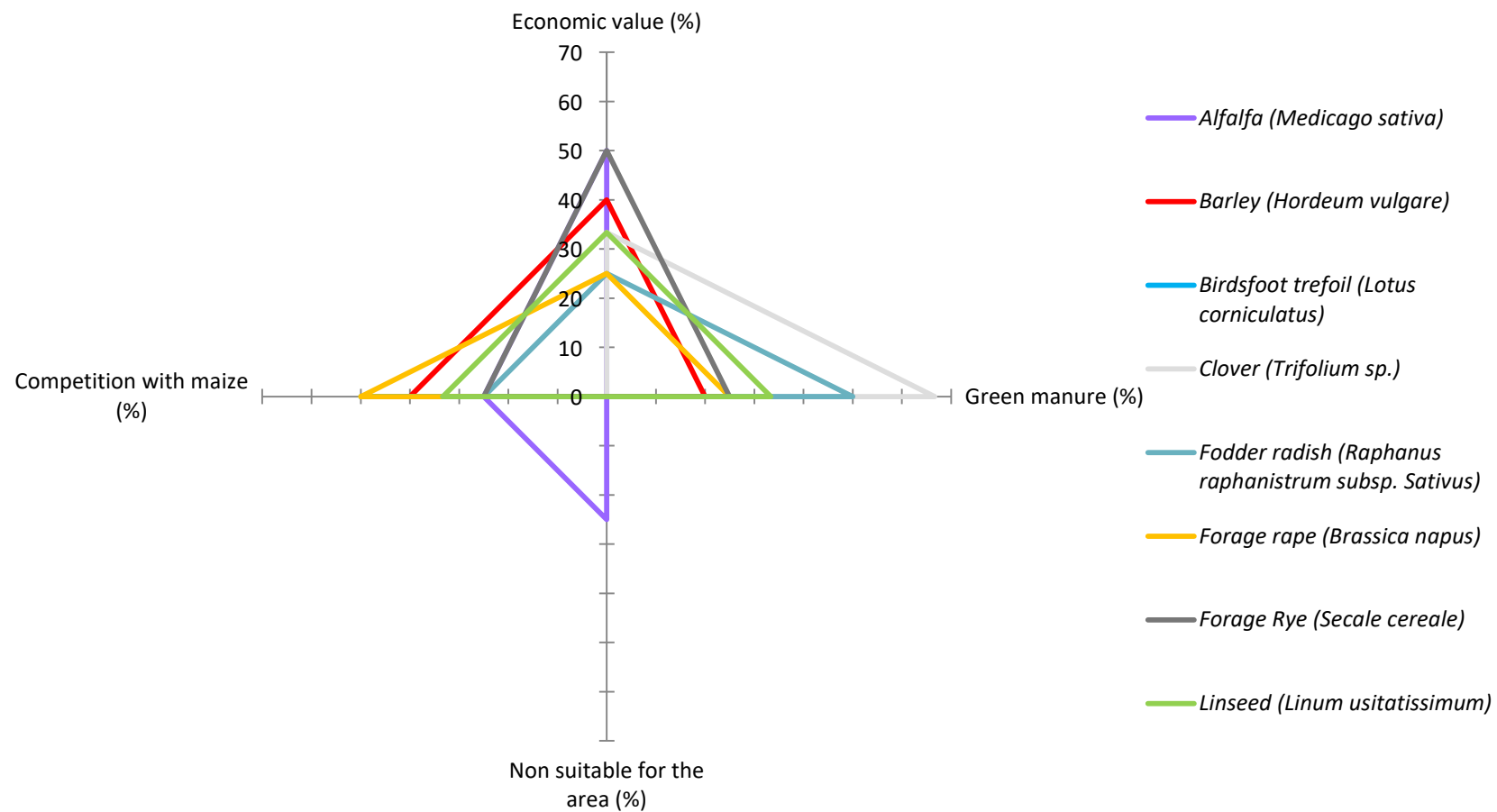


Figure 28 Comparison of responses to the question: why you sow/not sow any of the following with maize? For growers who did not experience erosion problems (n range = 2-10).

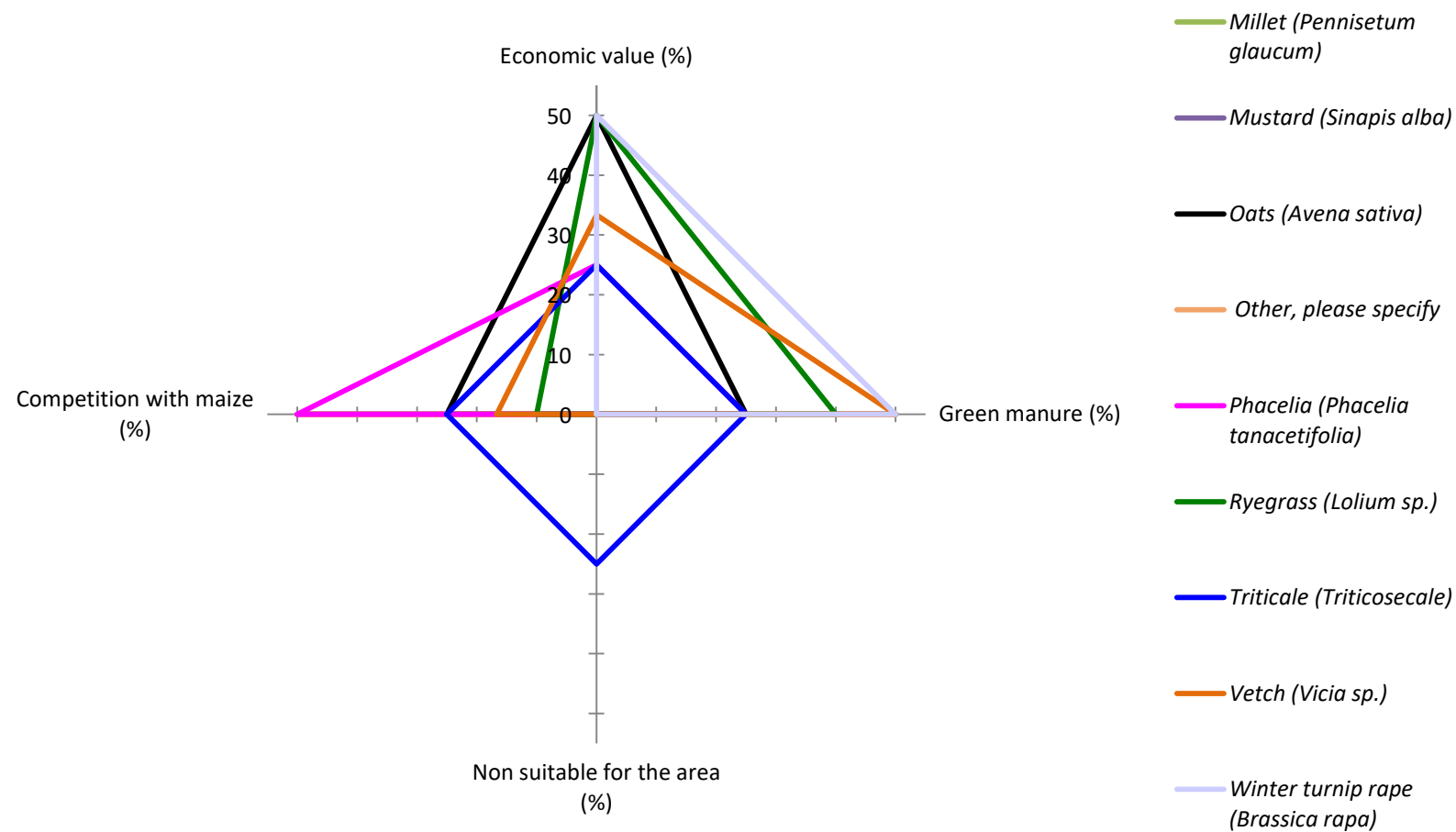


Figure 29 Comparison of responses to the question: why you sow/not sow any of the following with maize? For growers who did not experience erosion problems (n range = 2-10).

Growers were asked to choose whether they would undersow cover crops with maize or to plant post-harvest. There was not a clear preference for the majority of the cover crops listed; probably due to the low number of responses per question. On average, there were eight responses per cover crop species for the growers who experienced runoff and erosion problems and six responses for the growers who did not encounter runoff and erosion (Figures 30 and 31). Additionally, for half of the cover crop species listed the majority of the respondents chose the unsure/not applicable option.

Considering the growers who experienced erosion, for nine cover crop species over the 18 listed there was not a clear preference between sowing post-harvest and Undersowing. Forage rye (*Secale cereale*), ryegrass (*Lolium* sp.) and winter turnip rape (*Brassica rapa*) were the three species that grower would sow post-harvest., while vetch (*Vicia* sp.) was the only cover crop that growers would rather undersow than sow post-harvest (Figure 30). All growers who chose to add a cover crop of their choice in the column other (please specify) selected that they would sow post-harvest and in most cases the suggested cover crop was wheat (*Triticum aestivum*)

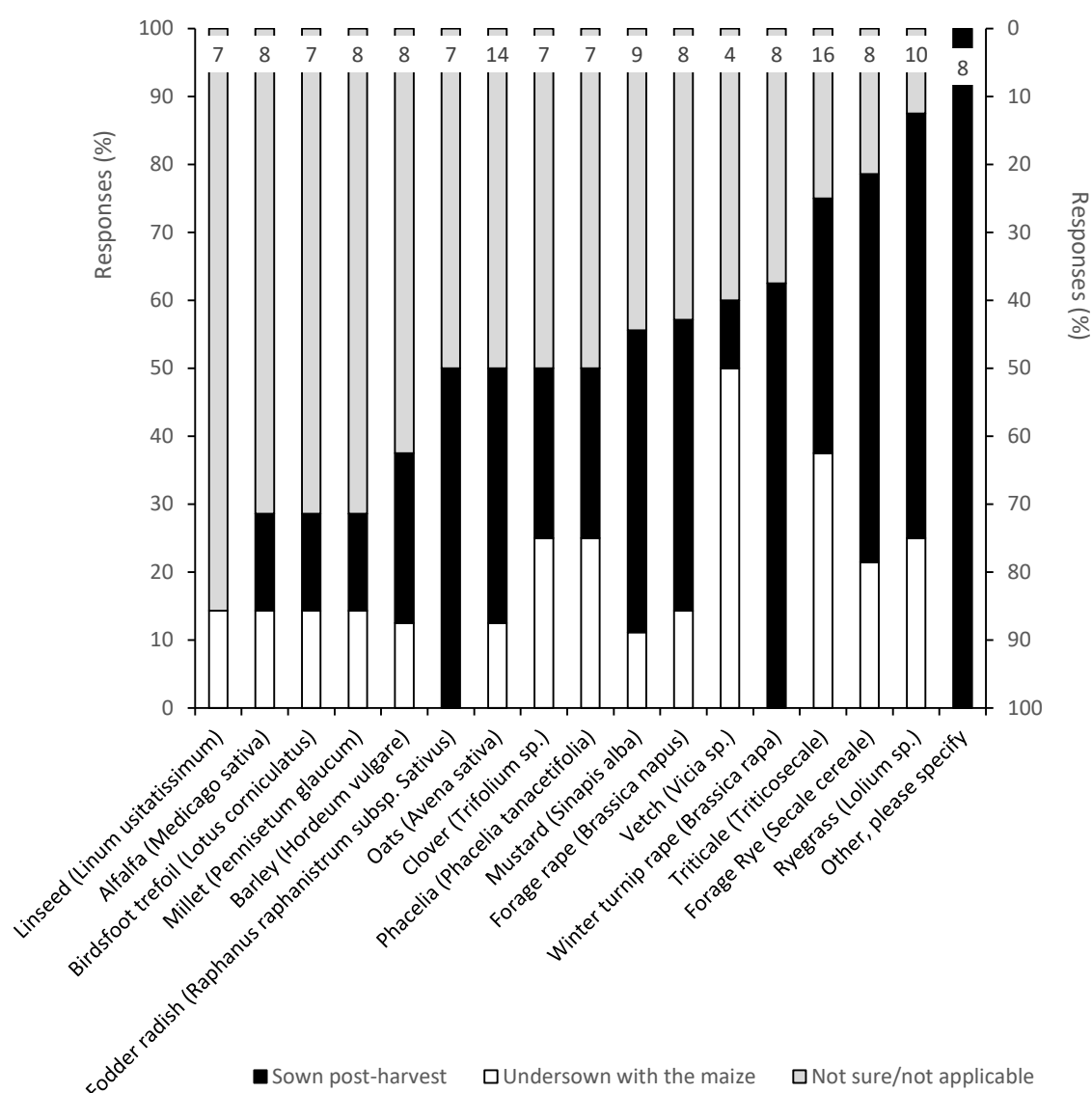


Figure 30 Comparison of sowing preferences considering growers who experienced erosion problems. The numbers on the bars represent the total number of responses per cover crop.

The majority of the growers who did not encounter runoff and soil erosion was not sure on how to sow the cover crop or did not want to plant cover crops. The majority would sow forage rye (*Secale cereale*) and ryegrass (*Lolium sp.*) post-harvest; while undersowing was only chose by less than 50% of the respondents.

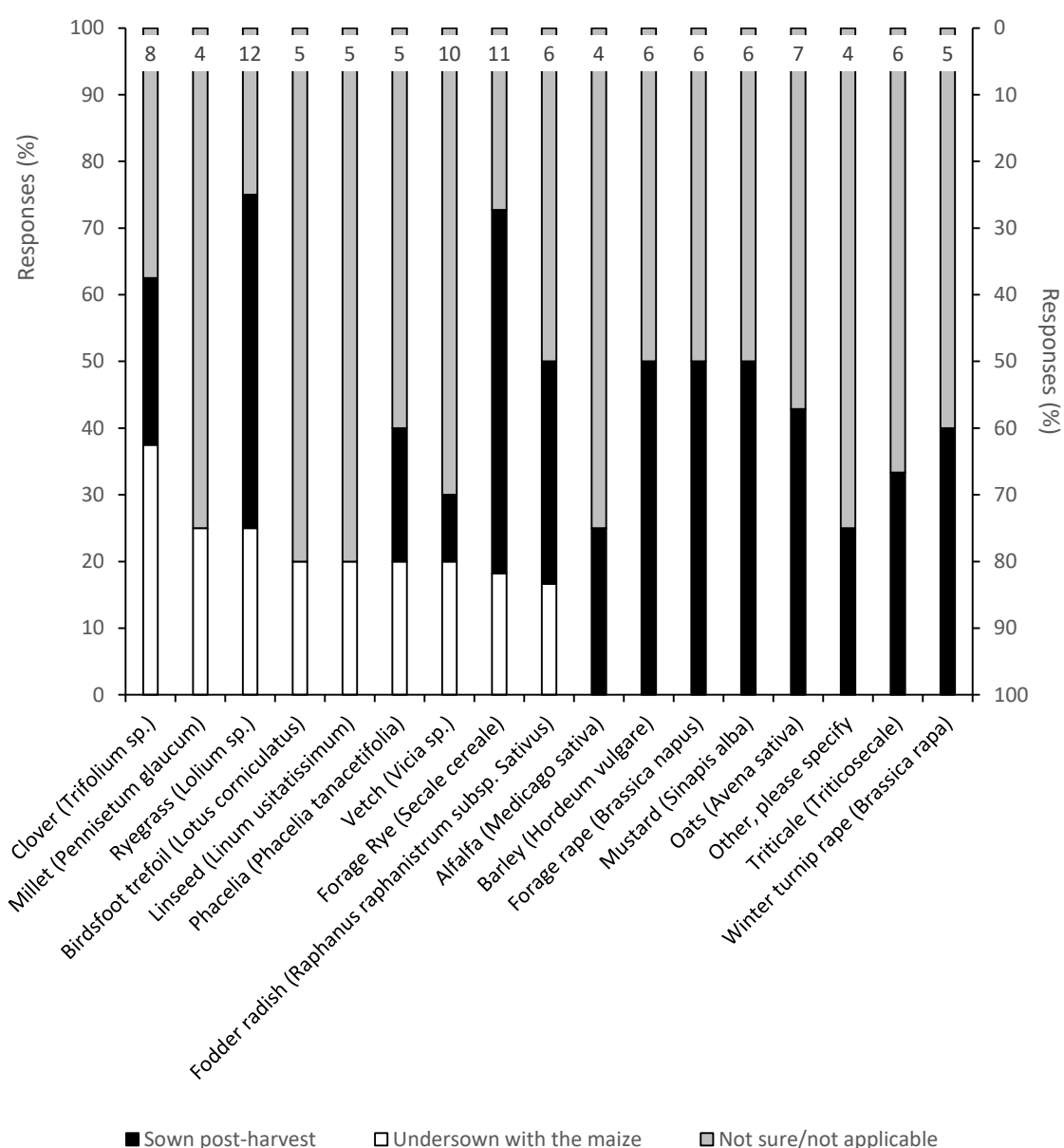


Figure 31 Comparison of sowing preferences considering growers who did not experience erosion problems. The numbers on the bars represent the total number of responses per cover crop.

The majority (70%) of the maize growers who experienced erosion were located in a catchment sensitive farming area in the South West of England (Figures 32 and 33). On the other hand, most of the growers who did not experience erosion (70%) were not in a catchment sensitive area and were distributed in

the different regions in the UK. However, the number of responses to these questions was low, therefore not representative of all the respondents.

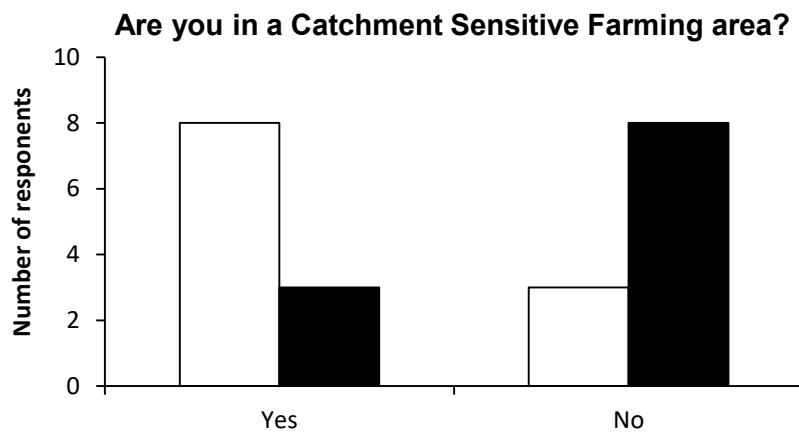


Figure 32 Comparison of responses to the question: are you in a Catchment Sensitive Farming area? Growers that experienced erosion (white). Growers that did not experience erosion (black).

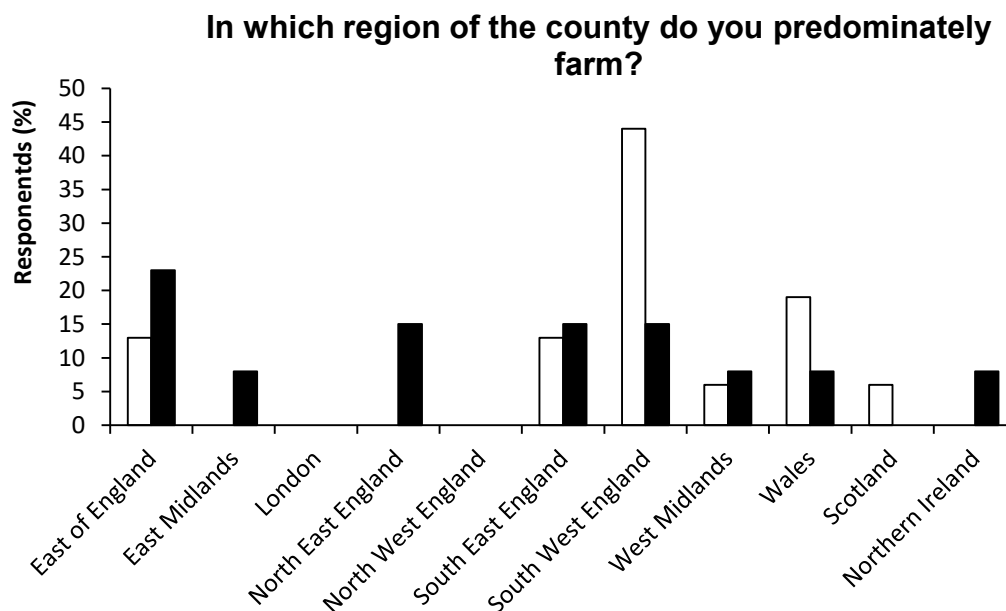


Figure 33 Comparison of locations where respondents predominantly farm. Growers that experienced erosion (white), n range=0-7. Growers that did not experience erosion (black), n range=0-3.

3.2 Experiment 1

The results presented in this section are consistent with the following hypothesis:

Planting Lolium multiflorum and Secale cereale during maize production will affect soil microbial community, reduce runoff, and control soil erosion compared to various conventional post-harvest methods.

3.2.1 Cover crops

Plant height and stem density were significantly different between *S. cereale* and *L. multiflorum*. *S. cereale* was 49% shorter than *L. multiflorum* drilled and 72% shorter than *L. multiflorum* broadcast ($P < 0.01$; Figure 34). *S. cereale* had 102% less stem density compared to *L. multiflorum* drilled and 104% less compared to *L. multiflorum* broadcast ($P < 0.05$; Figure 35). However, plant characteristics were not different between *L. multiflorum* drilled and broadcast.

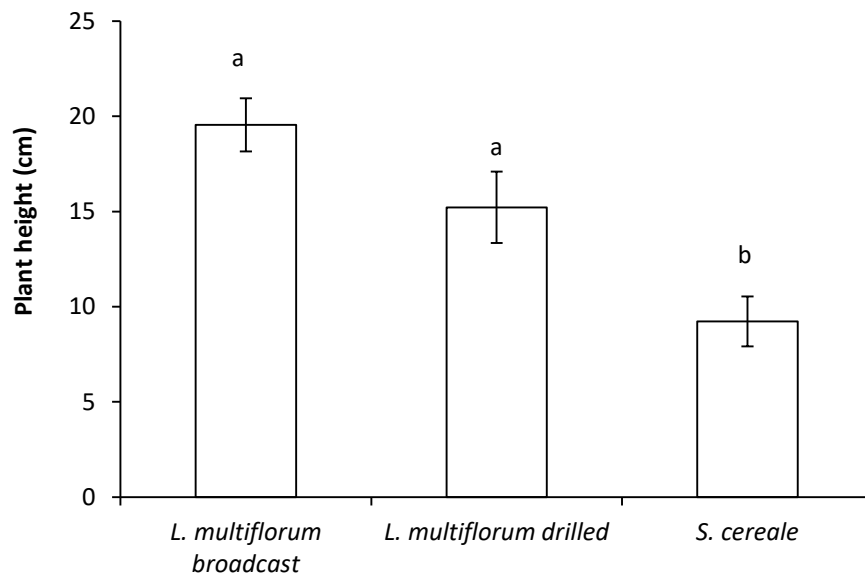


Figure 34 Comparison of plant height (cm) between cover crops treatments measured in March 2015. Vertical bars represent SE (n=3), letters represent homogenous groupings. *L. multiflorum* broadcast= *L. multiflorum* broadcast, mouldboard; *L. multiflorum* drilled= *L. multiflorum* drilled mouldboard; *S. cereale*= *S. cereale* drilled, mouldboard.

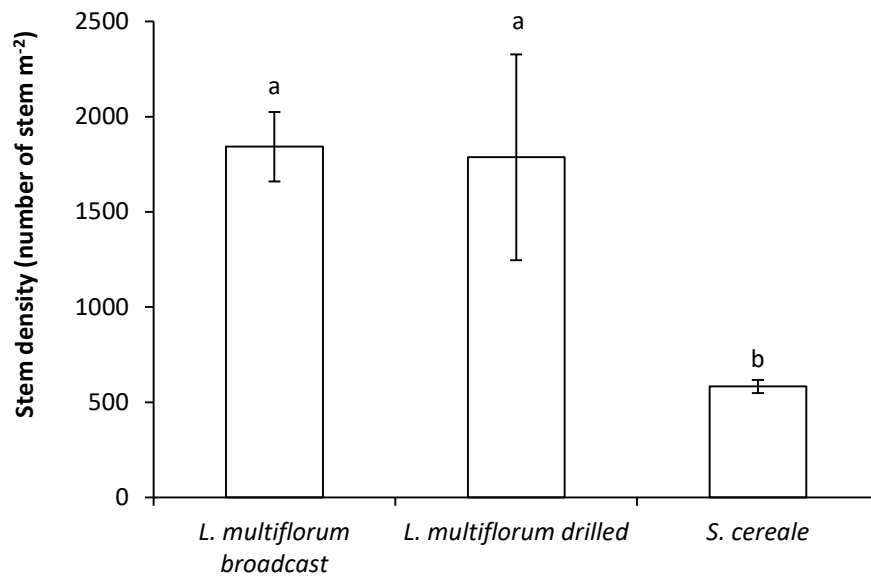


Figure 35 Comparison of stem density (number of stem m⁻²) between cover crops treatments measured in March 2015. Vertical bars represent SE (n=3), letters represent homogenous groupings. *L. multiflorum* broadcast= *L. multiflorum* broadcast, mouldboard; *L. multiflorum* drilled= *L. multiflorum* drilled mouldboard; *S. cereale*= *S. cereale* drilled, mouldboard.

3.2.2 Soil biology

3.2.2.1 Microbial biomass and microbial quotient

There was not a clear difference in MB between the non-cover crop treatments (in black Figure 36) and cover crop treatments (white). The non-cover crop treatments, the traditional, disc and cross-plough methods, had on average 30% larger MB compared to *L. multiflorum* broadcast ($P < 0.01$; Figure 36). However, there were no significant differences between the non-cover crop treatments traditional and disc method methods and the cover crop treatments *L. multiflorum* drilled and *S. cereale*.

In reference to the cover crop treatments, *L. multiflorum* drilled had 27% less MB compared to *L. multiflorum* broadcast and 29% less MB compared to *S. cereale*. *L. multiflorum* and *S. cereale* were not significantly different.

Additionally, there were no significant differences, between the non-cover crop treatments.

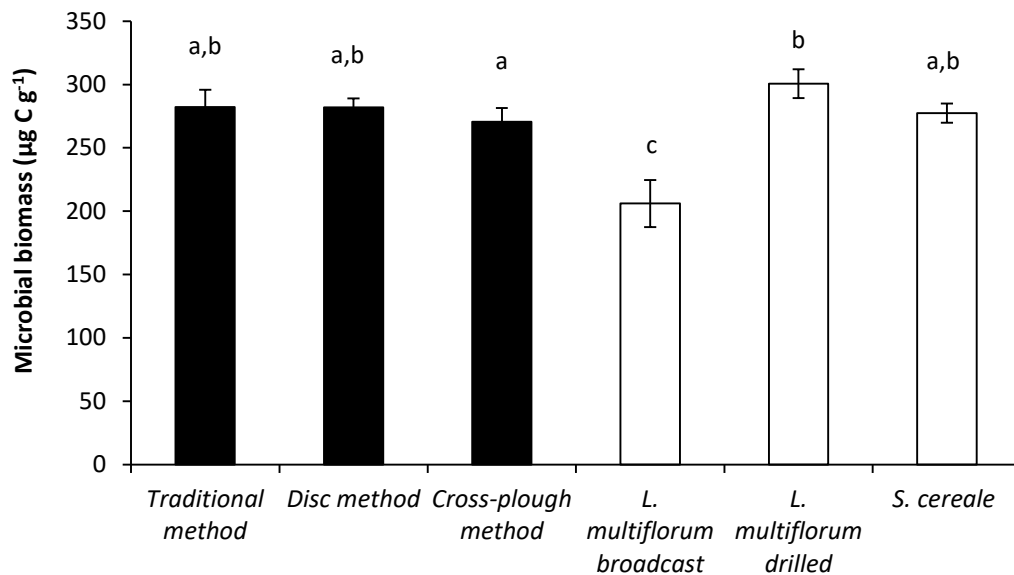


Figure 36 Comparison of microbial biomass ($\mu\text{g C g}^{-1}$) between cover crops treatments (white) and non-cover crops treatments (black) measured in March 2015. Vertical bars represent SE ($n=3$), letters represent homogenous groupings. Traditional method= No cover crops, mouldboard; Disc method= No cover crops, disc; Cross-plough method = No cover crops, mouldboard + chisel; *L. multiflorum* broadcast= *L. multiflorum* broadcast, mouldboard; *L. multiflorum* drilled= *L. multiflorum* drilled, mouldboard; *S. cereale*= *S. cereale* drilled, mouldboard.

There was not a significant difference between the non-cover crop treatments (in black Figure 37) and cover crop treatments (white) ($P>0.05$; Figure 37) and the average microbial quotient was similar across the treatments.

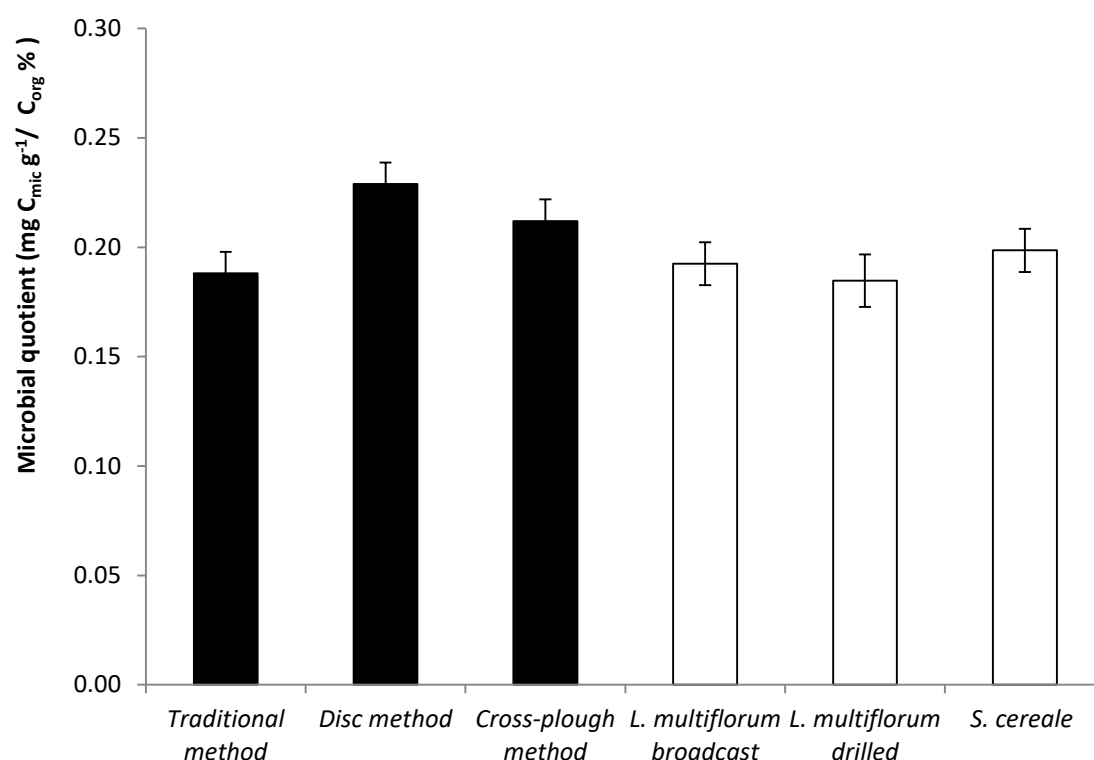


Figure 37 Comparison of microbial quotient (mg C_{mic} g⁻¹ / C_{org} %) between cover crop treatments (white) and the traditional method (black) in March 2015. Vertical bars represent SE (n=3). Traditional method= No cover crops, mouldboard; Disc method= No cover crops, disc; Cross-plough method = No cover crops, mouldboard + chisel; *L. multiflorum* broadcast= *L. multiflorum* broadcast, mouldboard; *L. multiflorum* drilled= *L. multiflorum* drilled, mouldboard; *S. cereale*= *S. cereale* drilled, mouldboard.

3.2.3 Soil chemistry

Imposed treatments significantly affect OM, TN, TC, TOC and AP, but it did not affect EC and pH (Table 17). There was a significant difference due to sampling date of sampling for EC; yet no other soil property was affected by sampling date. Additionally, sampling date x treatment effect was not significant on any measured soil properties (Table 17).

Table 17 Summary of soil chemistry ANOVA P values from Experiment 1.

Soil property	P values		
	Treatment	Sampling date	Treatment x Sampling date
OM	<0.01	>0.05	>0.05
TN	<0.001	>0.05	>0.05
TC	<0.001	>0.05	>0.05
TOC	<0.001	>0.05	>0.05
AP	<0.001	>0.05	>0.05
EC	>0.05	<0.001	>0.05
pH	>0.05	>0.05	>0.05

AP=Available Phosphorous; TN=Total Nitrogen; TC=Total Carbon; TOC=Total Organic Carbon; EC=Electrical Conductivity; OM=Organic matter.

3.2.3.1 Organic matter

There was not a marked difference in OM between the non-cover crop treatments (in black Figure 38) and cover crop treatments (white). The traditional method had 28% larger OM compared to *L. multiflorum* drilled and 11% larger compared to *L. multiflorum* broadcast ($P<0.01$; Figure 38). Similarly, the cross-plough method had 19% larger OM compared to *L. multiflorum* drilled. However, there were no significant differences between the traditional method and *S. cereale*, and between the cross-plough method, *L. multiflorum* broadcast

and *S. cereale*. Moreover, the disc method was similar to all cover crop treatments.

Considering the cover crop treatments, *L. multiflorum* drilled had 16% less OM compared to *L. multiflorum* broadcast and *S. cereale*; though no differences were found between *L. multiflorum* broadcast and *S. cereale*. In reference to the non-cover crops treatments, the disc method had 21% less OM compared to the traditional method and 12.5% less compared to the cross-plough method. Though, there were no significant differences between cross-plough method and the traditional method.

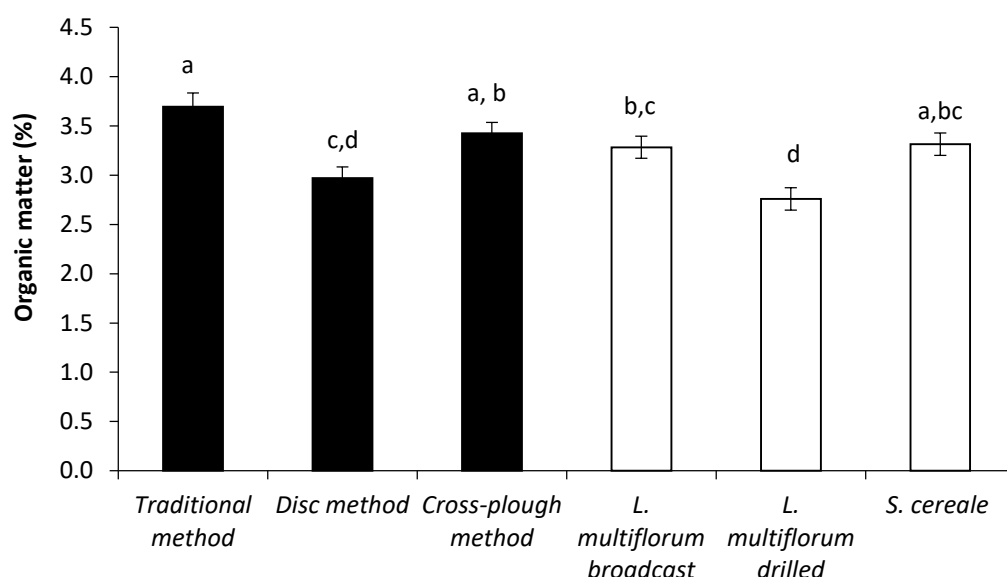


Figure 38 Comparison of organic matter (%) between cover crops treatments (white) and non-cover crops treatments (black) (two sampling points, December 2014 and March 2015). Vertical bars represent SE (n=6), letters represent homogenous groupings. Traditional method= No cover crops, mouldboard; Disc method= No cover crops, disc; Cross-plough method = No cover crops, mouldboard + chisel; *L. multiflorum* broadcast= *L. multiflorum* broadcast, mouldboard; *L. multiflorum* drilled= *L. multiflorum* drilled, mouldboard; *S. cereale*= *S. cereale* drilled, mouldboard.

3.2.3.2 Total Nitrogen

On average, the non-cover crop treatments (in black Figure 39) had larger TN than the cover crop treatments (in white). The non-cover crop treatments had 30% larger (average) TN compared to *L. multiflorum* drilled and the traditional and the cross-plough methods had 14% larger TN compared to *L. multiflorum* broadcast, and 7% larger compared to *S. cereale* ($P < 0.001$; Figure 39). However, there were no significant differences between the disc method, *L. multiflorum* broadcast and *S. cereale*.

Considering the cover crop treatments, *L. multiflorum* drilled had 17% less TN compared to *L. multiflorum* broadcast and 24% less compared to *S. cereale*. Although, there were no significant differences between *L. multiflorum* broadcast and *S. cereale*. In reference to the non-cover crop treatments, the disc method had 14% less TN compared to the traditional method and the cross-plough method. However, there were no significant differences between the traditional method and the cross-plough method.

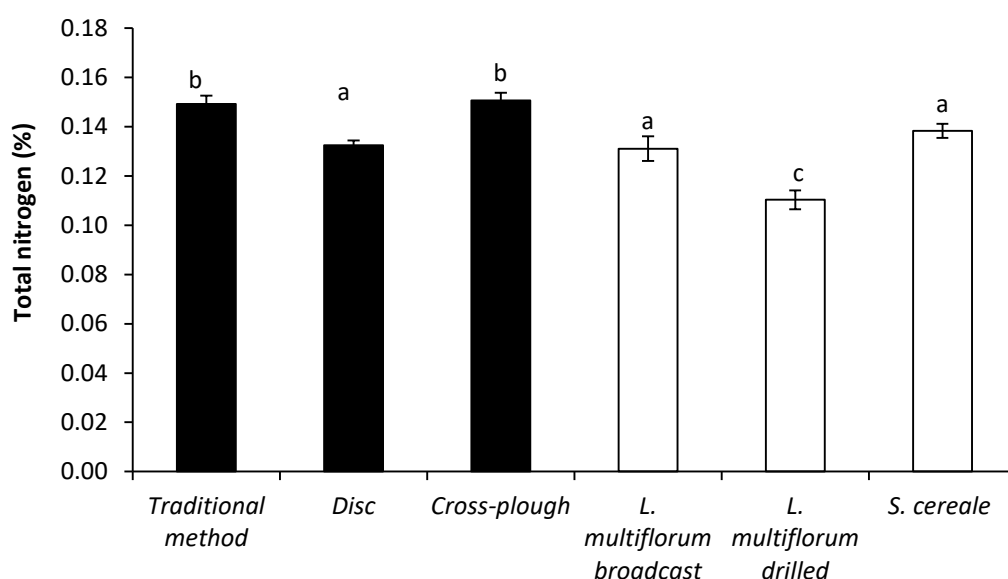


Figure 39 Comparison of total nitrogen (%) between cover crops treatments (white) and non-cover crops treatments (black) (two sampling points, December 2014 and March 2015). Vertical bars represent SE (n=6), letters represent homogenous groupings. Traditional method= No cover crops, mouldboard; Disc method= No cover crops, disc; Cross-plough method = No cover crops, mouldboard + chisel; *L. multiflorum* broadcast= *L. multiflorum* broadcast, mouldboard; *L. multiflorum* drilled= *L. multiflorum* drilled, mouldboard; *S. cereale*= *S. cereale* drilled, mouldboard.

3.2.3.3 Total Organic carbon

On average the non-cover crop treatments (in black Figure 40) had larger TOC than the cover crop treatments (white). The traditional method had 23% larger TOC compared to *L. multiflorum* broadcast, 41% larger compared to *multiflorum* drilled and 15% larger compared to *S. cereale* ($P < 0.001$; Figure 40). Similarly, the cross-plough method had 25% larger TOC compared to *L. multiflorum* broadcast, 43% larger compared to *L. multiflorum* drilled and 17% larger compared to *S. cereale*. On the contrary, the disc method had 15% larger TOC compared to *L. multiflorum* drilled, but it had 11% less compared to *S. cereale*.

Considering the cover crop treatments, *L. multiflorum* drilled had 18% less TOC compared to *L. multiflorum* broadcast and 26% less compared to *S. cereale*.

Although, *S. cereale* and *L. multiflorum* broadcast were not significantly different. In reference to the non-cover crop treatments, the disc method had 26% less TOC compared to the traditional method and 28% less compared to the cross-plough method. However, there were no significant differences between the traditional method and the cross-plough method.

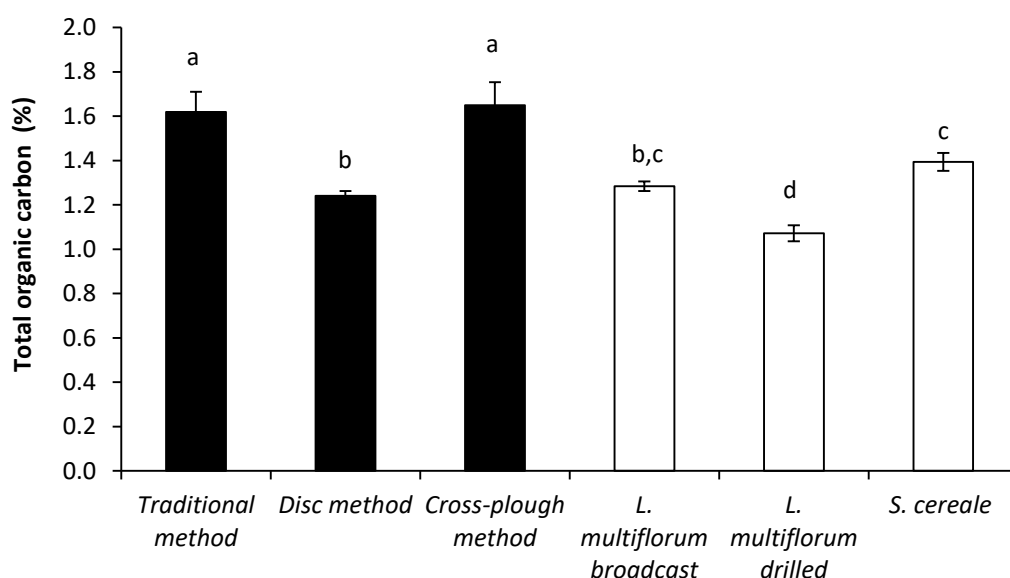


Figure 40 Comparison of total organic carbon (%) between post-harvest cover crops treatments (white) and non-cover crops treatments (black) (two sampling points, December 2014 and March 2015). Vertical bars represent SE (n=6), letters represent homogenous groupings. Traditional method= No cover crops, mouldboard; Disc method= No cover crops, disc; Cross-plough method = No cover crops, mouldboard + chisel; *L. multiflorum* broadcast= *L. multiflorum* broadcast, mouldboard; *L. multiflorum* drilled= *L. multiflorum* drilled, mouldboard; *S. cereale*= *S. cereale* drilled, mouldboard.

3.2.3.4 Available Phosphorus

There was not a constant difference in AP between the non-cover crop treatments (in black Figure 38) and cover crop treatments (white). The traditional and the cross-plough methods had 69% larger AP compared to *L.*

multiflorum broadcast and 86% larger compared to *L. multiflorum* drilled ($P < 0.001$; Figure 31). However, they were not different compared to *S. cereale* and there were no significant differences between the disc method, *L. multiflorum* broadcast and *L. multiflorum* drilled.

In reference to the cover crop treatments, *L. multiflorum* drilled had 81% less AP compared to *S. cereale*. However, there were no significant differences between *L. multiflorum* broadcast and *L. multiflorum* drilled. Considering, the non-cover crop treatments, the disc method had 62% less AP compared to the traditional method and 62% less compared to the cross-plough method. However, there were no differences between the cross-plough method and the traditional method.

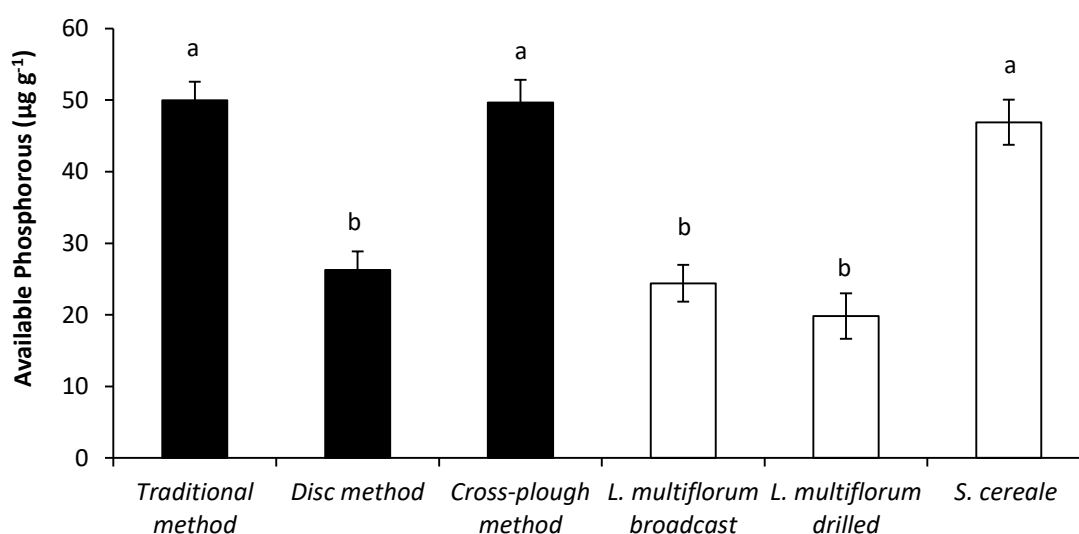


Figure 41 Comparison of available phosphorus ($\mu\text{g g}^{-1}$) between cover crops treatments (white) and non-cover crops treatments (black) (two sampling points, December 2014 and March 2015). Vertical bars represent SE ($n=6$), letters represent homogenous groupings. Traditional method= No cover crops, mouldboard; Disc method= No cover crops, disc; Cross-plough method = No cover crops, mouldboard + chisel; *L. multiflorum* broadcast= *L. multiflorum* broadcast, mouldboard; *L. multiflorum* drilled= *L. multiflorum* drilled, mouldboard; *S. cereale*= *S. cereale* drilled, mouldboard.

3.2.3.5 Soil pH and electrical conductivity

There was not a marked difference in pH and EC₁₋₅ between the non-cover crop treatments (in black Figures 42 and 43) and cover crop treatments (white). Soil pH was not significantly affected either by treatments (Figure 42) or by sampling date ($P>0.05$). Similarly, EC₁₋₅ was not affected by treatments ($P>0.05$; Figure 43). However, EC₁₋₅ decreased by 24% from December 2014 to March 2015 ($P<0.001$; Figure 44). Average EC₁₋₅ measured between December 2014 and March 2015 was $54.4 \pm 2.0 \mu\text{S cm}^{-1}$, which corresponds to an EC_{sa} of 0.58 dS m⁻¹.

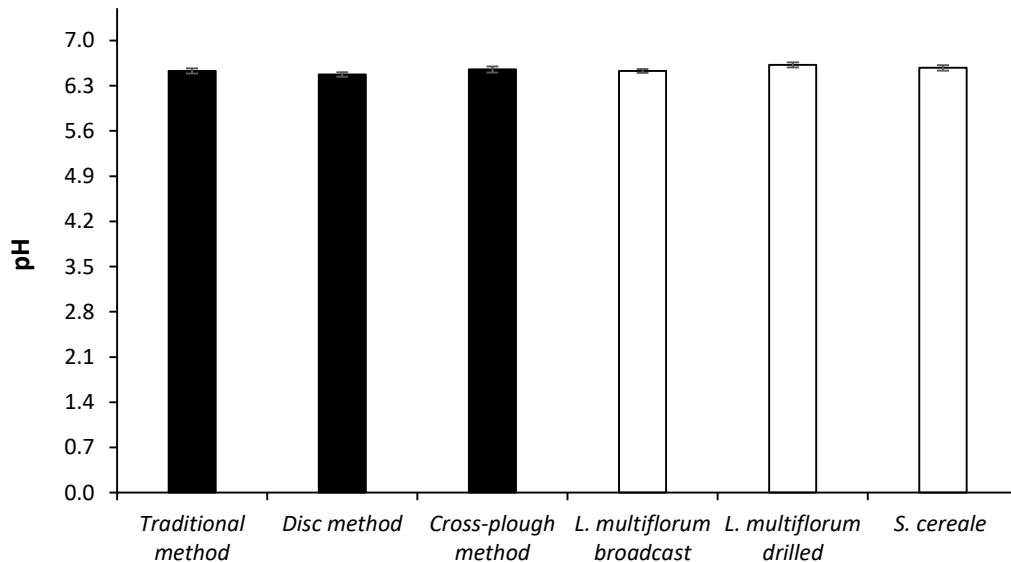


Figure 42 Comparison of pH between cover crops treatments (white) and non-cover crops treatments (black) (two sampling points, December 2014 and March 2015). Vertical bars represent SE (n=6). Traditional method= No cover crops, mouldboard; Disc method= No cover crops, disc; Cross-plough method = No cover crops, mouldboard + chisel; *L. multiflorum* broadcast= *L. multiflorum* broadcast, mouldboard; *L. multiflorum* drilled= *L. multiflorum* drilled, mouldboard; *S. cereale*= *S. cereale* drilled, mouldboard.

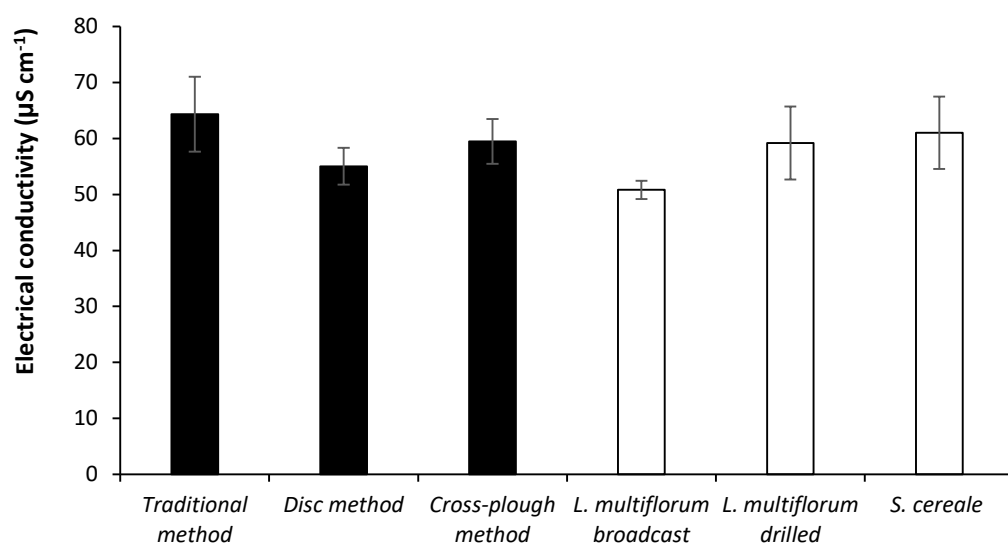


Figure 43 Comparison of electrical conductivity ($\mu\text{S cm}^{-1}$) between cover crops treatments (white) and non-cover crops treatments (black) (two sampling points, December 2014 and March 2015). Vertical bars represent SE (n=6). Traditional method= No cover crops, mouldboard; Disc method= No cover crops, disc; Cross-plough method= No cover crops, mouldboard + chisel; *L. multiflorum* broadcast= *L. multiflorum* broadcast, mouldboard; *L. multiflorum* drilled= *L. multiflorum* drilled mouldboard; *S. cereale*= *S. cereale* drilled, mouldboard.

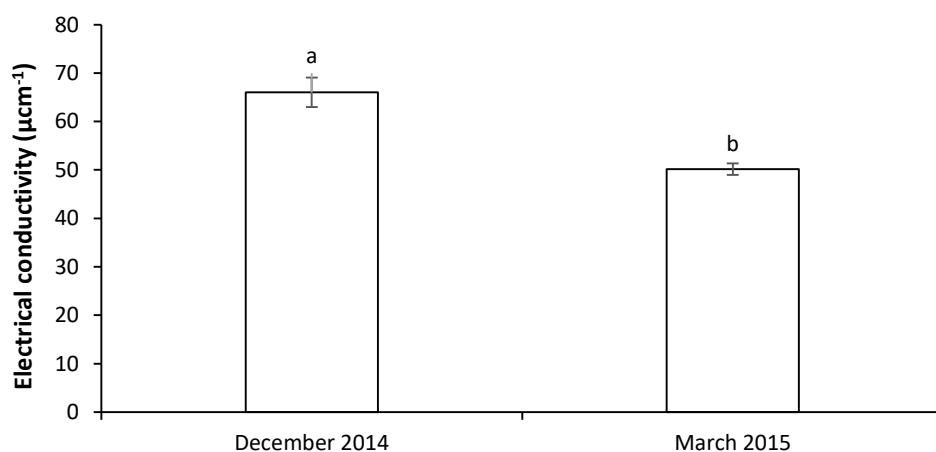


Figure 44 Comparison of soil electrical conductivity ($\mu\text{S cm}^{-1}$) in December 2014 and March 2015. Vertical bars represent SE (n=18), letters represent homogenous groupings.

3.2.4 Soil physics

3.2.4.1 Bulk density

Soil bulk density was not significantly different between the treatments ($P>0.05$; Figure 35) and there was not a discernible difference in bulk density between the non-cover crop treatments (in black) and cover crop treatments (white; Figure 45).

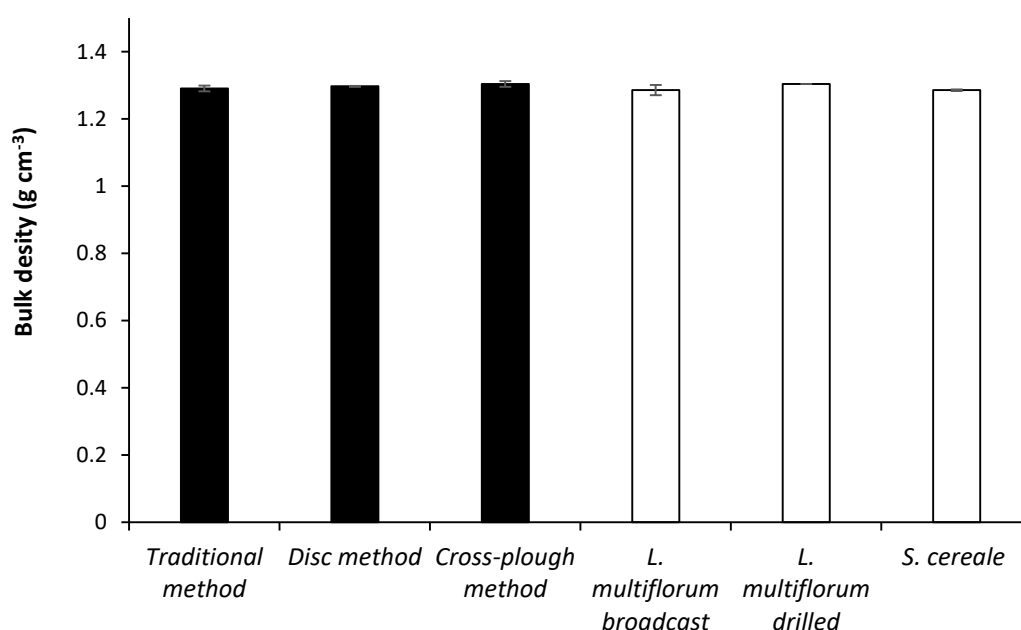


Figure 45 Comparison of bulk density (g cm⁻³) between cover crops treatments (white) and non-cover crops treatments (black) measured in March 2015. Vertical bars represent SE (n=3). Traditional method= No cover crops, mouldboard; Disc method= No cover crops, disc; Cross-plough method = No cover crops, mouldboard + chisel; *L. multiflorum* broadcast= *L. multiflorum* broadcast, mouldboard; *L. multiflorum* drilled= *L. multiflorum* drilled, mouldboard; *S. cereale*= *S. cereale* drilled, mouldboard.

3.2.4.2 Runoff and soil loss

Runoff, nutrients in the runoff (nitrates, ammonium and phosphates) and soil loss were not significantly different between non-cover crop treatments and cover crops for any collection period or cumulative values ($P>0.05$; Figures 46-

52). No significant differences were found between any of the treatments ($P>0.05$; Figures 46-52).

There was no significant difference in runoff volume between the treatments during the first collection period 18/12/2014 - 13/01/2015 (Figure 46). During this collection period, in the cross-plough treatment and *L. multiflorum* drilled no runoff was collected. On average the traditional method had the greatest runoff production. However, due to the great deviation (SE) was observed within the treatments the difference was not significant.

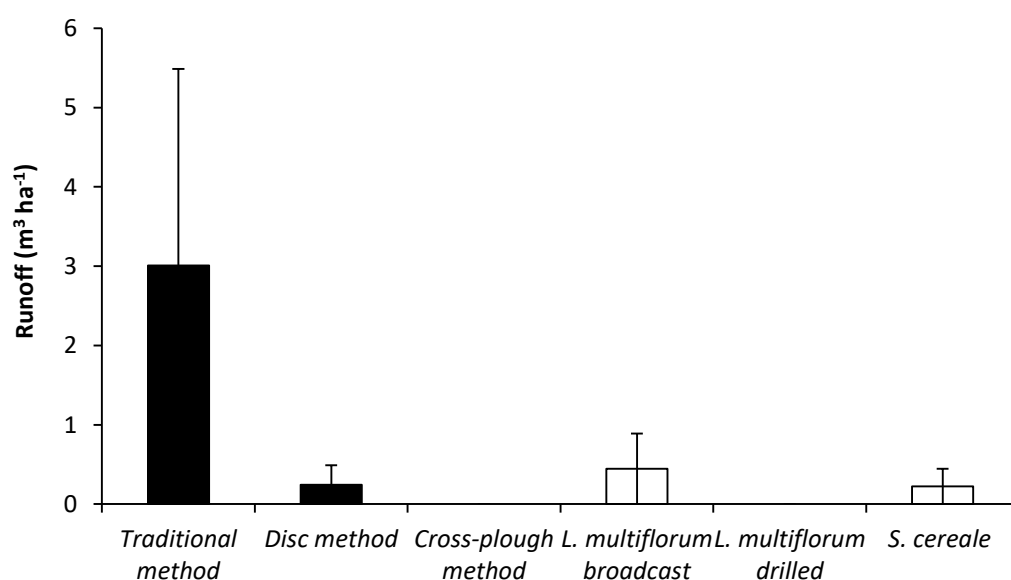


Figure 46 Comparison of runoff ($\text{m}^3 \text{ ha}^{-1}$) between post-harvest cover crops treatments (white) and non-cover crops treatments (black) collected between 18/12/2014 and 13/01/2015. Vertical bars represent SE ($n=3$). Traditional method= No cover crops, mouldboard; Disc method= No cover crops, disc; Cross-plough method= No cover crops, mouldboard + chisel; *L. multiflorum* broadcast= *L. multiflorum* broadcast, mouldboard; *L. multiflorum* drilled= *L. multiflorum* drilled mouldboard; *S. cereale*= *S. cereale* drilled, mouldboard.

There was no significant difference in runoff volume between the treatments during the second collection period 22/01/2015 -15/03/2015 (Figure 47). During

this period all the treatments produced runoff. On average the traditional method had the greatest runoff production and *L. multiflorum* the least. However, the differences were not significant due to the great deviation (SE) recorded within the treatments. Among the non-cover crop treatments the disc method and the cross-plough method had similar average levels of runoff.

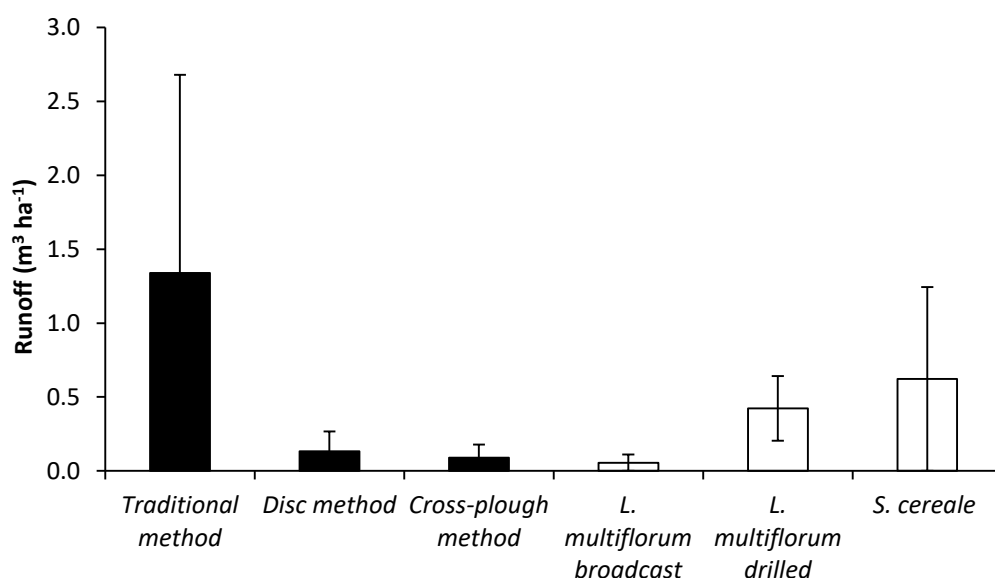


Figure 47 Comparison of runoff ($\text{m}^3 \text{ha}^{-1}$) between cover crops treatments (white) and non-cover crops treatments (black) collected between 22/01/2015 and 15/03/2015. Vertical bars represent SE ($n=3$). Traditional method= No cover crops, mouldboard; Disc method= No cover crops, disc; Cross-plough method= No cover crops, mouldboard + chisel; *L. multiflorum* broadcast= *L. multiflorum* broadcast, mouldboard; *L. multiflorum* drilled= *L. multiflorum* drilled mouldboard; *S. cereale*= *S. cereale* drilled, mouldboard.

There was no significant difference in runoff volume accumulated over the two collection periods (cumulative runoff; Figure 48). On average the traditional method had the greatest runoff production and *L. multiflorum* broadcast the least. However, the differences were not significant due to the great deviation (SE) recorded within the treatments. Among the non-cover crop treatments the

disc method and the cross-plough method had similar average levels of runoff. In regards to the cover crop treatments *L. multiflorum* drilled and *S. cereale* had produced similar average quantities.

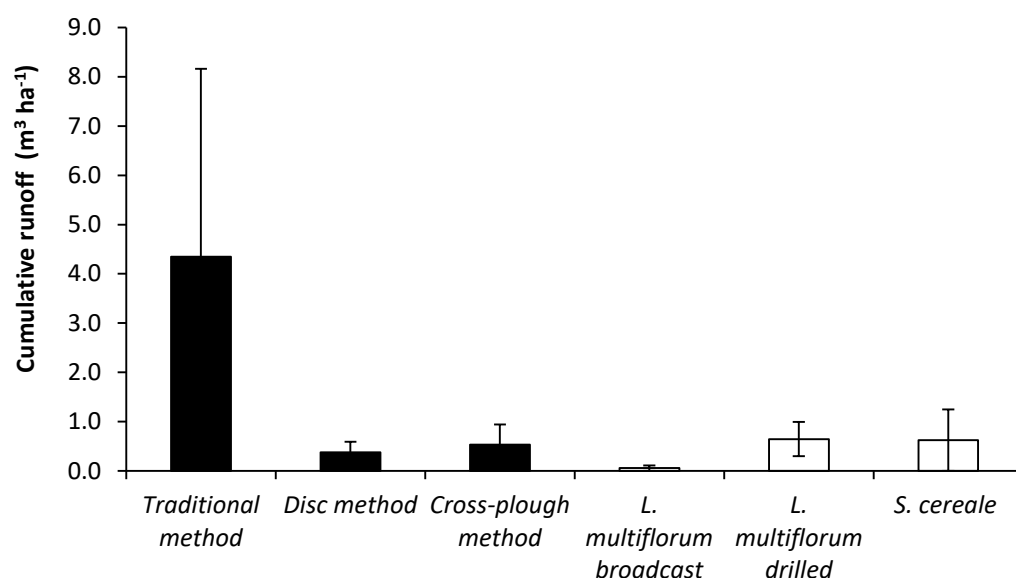


Figure 48 Comparison of cumulative runoff ($\text{m}^3 \text{ha}^{-1}$) between cover crops treatments (white) and non-cover crops treatments (black) collected between the two sampling periods. Vertical bars represent SE ($n=6$). Traditional method= No cover crops, mouldboard; Disc method= No cover crops, disc; Cross-plough method= No cover crops, mouldboard + chisel; *L. multiflorum* broadcast= *L. multiflorum* broadcast, mouldboard; *L. multiflorum* drilled= *L. multiflorum* drilled mouldboard; *S. cereale*= *S. cereale* drilled, mouldboard.

Nutrient loss in the runoff and nutrient levels in the runoff were only measured during the second collection period (22/01/2015 -15/03/2015). No significant differences were found ($P>0.05$; Figures 49-52).

The level of ammonium measured in the runoff was not significantly different between the treatments ($P>0.05$; Figure 49) due to the great difference (SE) in runoff volume within the treatments. On average the cross-plough method had the greatest level of ammonium in the runoff and traditional method the least.

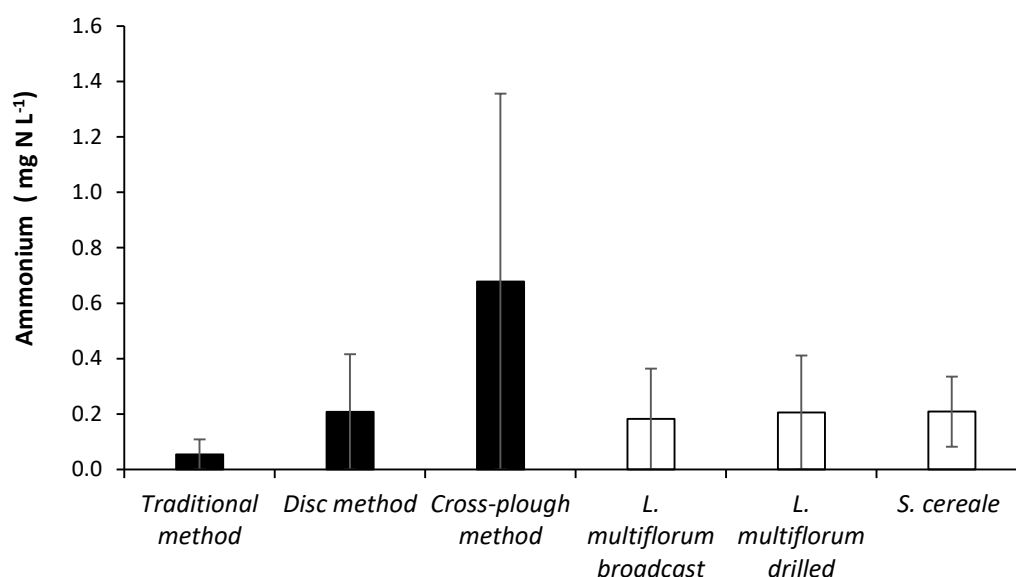


Figure 49 Comparison of ammonium content in the runoff (mg N L⁻¹) between cover crops treatments (white) and non-cover crops treatments (black) collected between 22/01/2015 and 15/03/2015. Vertical bars represent SE (n=3). Traditional method= No cover crops, mouldboard; Disc method= No cover crops, disc; Cross-plough method= No cover crops, mouldboard + chisel; *L. multiflorum* broadcast= *L. multiflorum* broadcast, mouldboard; *L. multiflorum* drilled= *L. multiflorum* drilled mouldboard; *S. cereale*= *S. cereale* drilled, mouldboard.

Similarly, the level of total oxidised nitrogen measured in the runoff collected during the second collection period (22/01/2015 -15/03/2015) was not significantly different between the treatments ($P>0.05$; Figure 50). In this case the average level of total oxidised nitrogen measured was similar across the treatments and SE was greater than the average for all treatments a part of *S. cereale*.

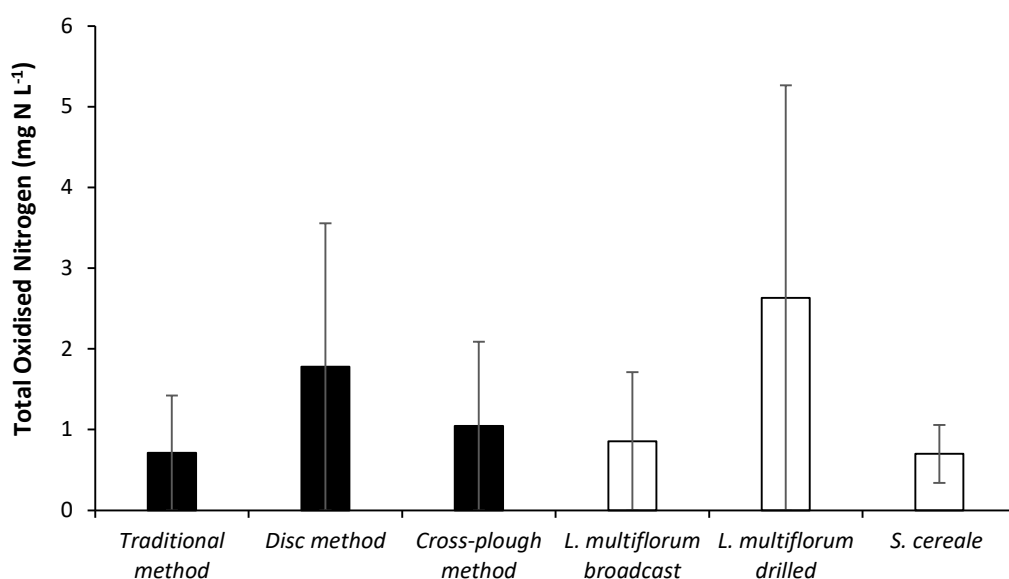


Figure 50 Comparison of total oxidised nitrogen content in the runoff (mg N L⁻¹) between post-harvest cover crops treatments (white) and non-cover crops treatments (black) collected between 22/01/2015 and 15/03/2015. Vertical bars represent SE (n=3). Traditional method= No cover crops, mouldboard; Disc method= No cover crops, disc; Cross-plough method= No cover crops, mouldboard + chisel; *L. multiflorum* broadcast= *L. multiflorum* broadcast, mouldboard; *L. multiflorum* drilled= *L. multiflorum* drilled mouldboard; *S. cereale*= *S. cereale* drilled, mouldboard.

The level of phosphates measured in the runoff collected during the second collection period (22/01/2015 -15/03/2015) was not significantly different between the treatments ($P>0.05$; Figure 51), due to the great variability (SE) in runoff within the treatments. The average level of phosphates measured was similar across the treatments.

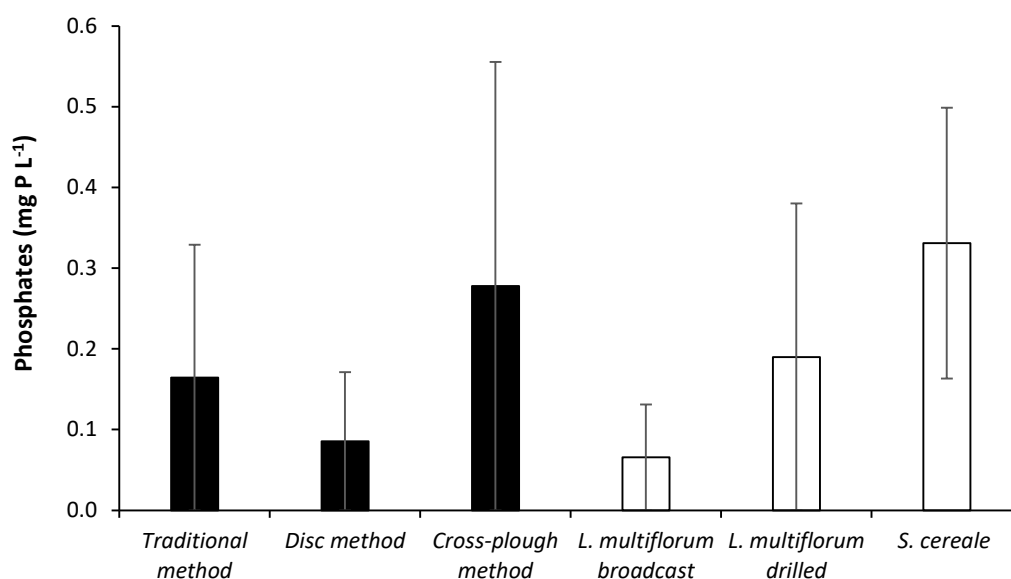


Figure 51 Comparison of phosphates content in the runoff (mg P L⁻¹) between cover crops treatments (white) and non-cover crops treatments (black) collected between 22/01/2015 and 15/03/2015. Vertical bars represent SE (n=6). Traditional method= No cover crops, mouldboard; Disc method= No cover crops, disc; Cross-plough method= No cover crops, mouldboard + chisel; *L. multiflorum* broadcast= *L. multiflorum* broadcast, mouldboard; *L. multiflorum* drilled= *L. multiflorum* drilled mouldboard; *S. cereale*= *S. cereale* drilled, mouldboard.

Soil loss was only measured during the second collection period (22/01/2015 - 15/03/2015). No significant differences were observed between the treatments ($P > 0.05$; Figure 52). On average the traditional method had the greatest soil loss and *L. multiflorum* broadcast the least. However, the differences were not significant due to the great deviation (SE) recorded within the treatments. Among the non-cover crop treatments the disc method and the cross-plough method had similar average levels of soil loss. In regards to the cover crop treatments *L. multiflorum* drilled and *S. cereale* had produced similar average quantities.

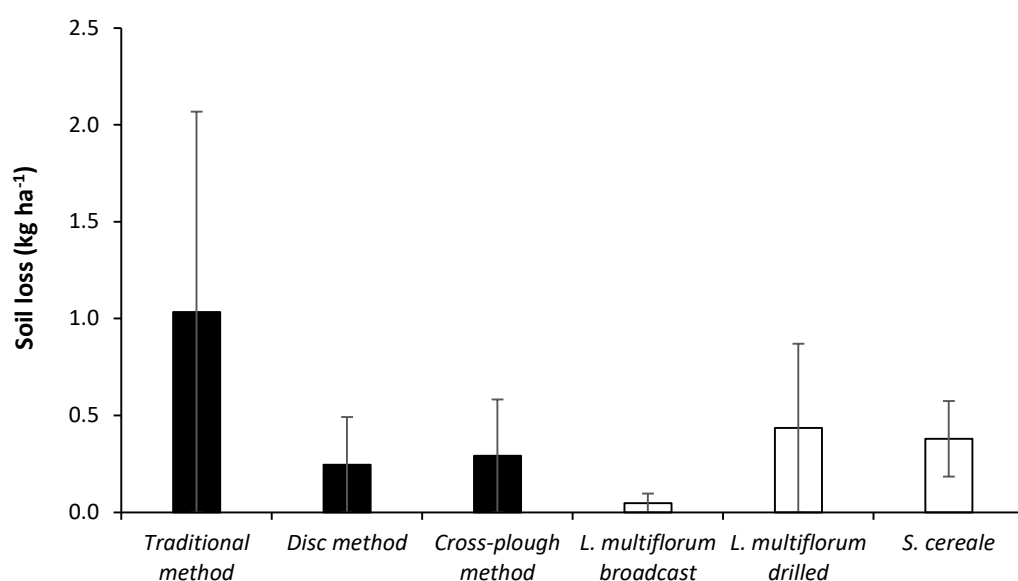


Figure 52 Comparison of soil loss (kg ha⁻¹) between cover crops treatments (white) and non-cover crops treatments (black) collected between 22/01/2015 and 15/03/2015. Vertical bars represent SE (n=3). Traditional method= No cover crops, mouldboard; Disc method= No cover crops, disc; Cross-plough method= No cover crops, mouldboard + chisel; *L. multiflorum* broadcast= *L. multiflorum* broadcast, mouldboard; *L. multiflorum* drilled= *L. multiflorum* drilled mouldboard; *S. cereale*= *S. cereale* drilled, mouldboard.

3.2.5 Soil texture

Average clay content was $16 \pm 0.9\%$ ($\pm$ SE), silt $62.8 \pm 2.7\%$ and sand $21.1 \pm 2.5\%$, which corresponds to sandy silt loam according to the UK soil textural triangle. No significant differences were found between the treatments for any of the texture classes ($P < 0.05$).

3.3 Experiment 2

The results presented in this section are consistent with the following hypotheses:

Using Lolium multiflorum cover crop and the cover crops mixtures L. multiflorum & T. alexandrinum and L. multiflorum & V. villosa during maize production will affect soil microbial community, reduce runoff, and control soil erosion compared to various conventional post-harvest methods. Additionally, cover crops will increase maize yield and the cover crop mixtures will have a more significant effect on soil microbiology than L. multiflorum alone.

3.3.1 Cover crop above-ground biomass and canopy cover

Cover crop above-ground biomass was not significantly different between *L. multiflorum* and the mixtures *L. multiflorum* & *T. alexandrinum* and *L. multiflorum* & *V. villosa* ($P > 0.05$; Figure 53). Similarly, cover crop canopy cover was not significantly affected by treatment ($P > 0.05$; Figure 54). However, there was a 16% increase in canopy cover between November 2015 and March 2016 (Figure 55), while location (area A, B and C) did not affect any parameter ($P > 0.05$).

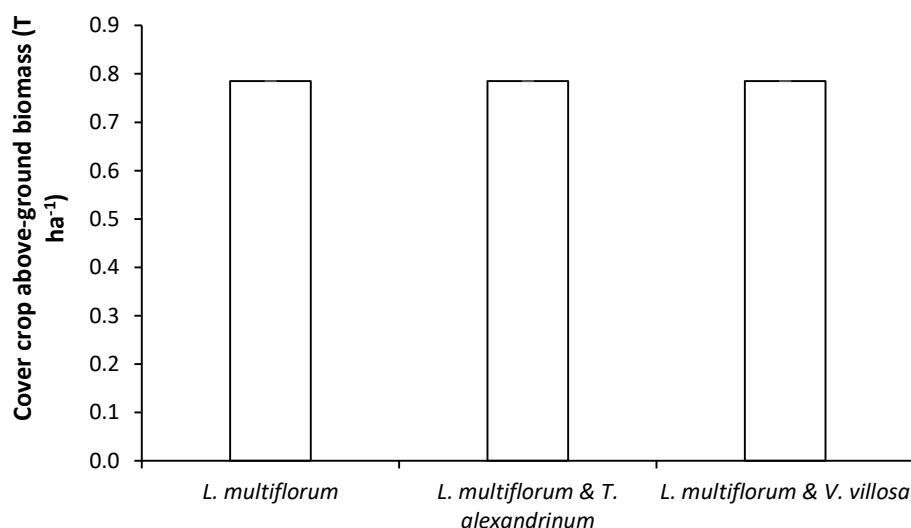


Figure 53 Comparison of yield (T ha⁻¹) between the cover crop treatments measured in March 2016. Vertical bars represent SE (n=5).

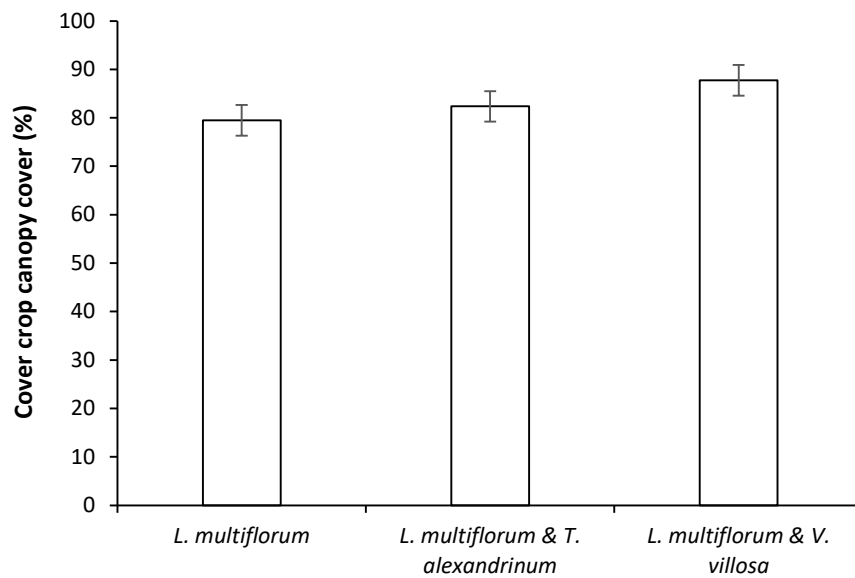


Figure 54 Comparison of canopy cover (%) between the cover crop treatments measured, two sampling points November 2015 and March 2016. Vertical bars represent SE (n=10).

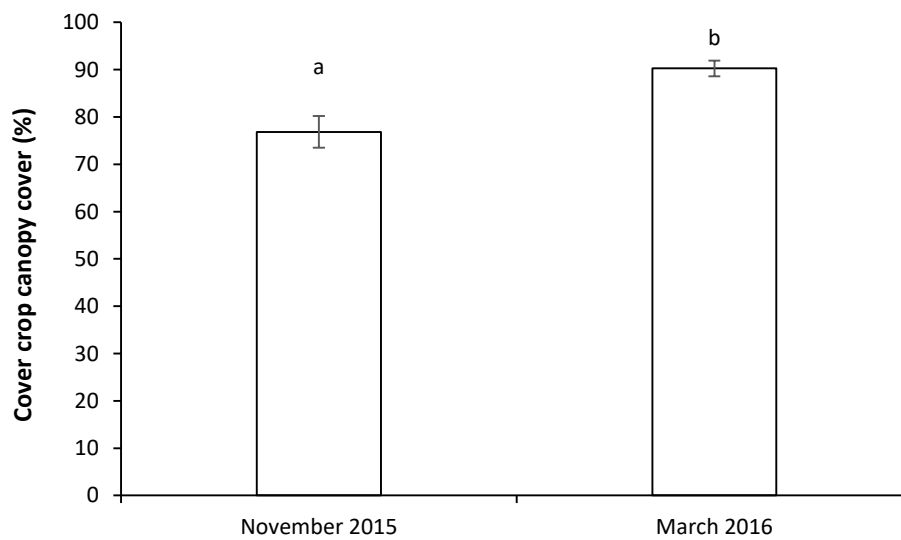


Figure 55 Comparison of canopy cover (%) between November 2015 and March 2016. Vertical bars represent SE (n=20), letters represent homogenous groupings.

3.3.2 Maize dry yield

The presence of cover crops did not significantly affect maize yield and there were not significant differences between the treatments ($P>0.05$; Figure 56) and locations ($P>0.05$). On average maize undersown with *L. multiflorum* & *T. alexandrinum* had lower yield; however, the difference was not significant.

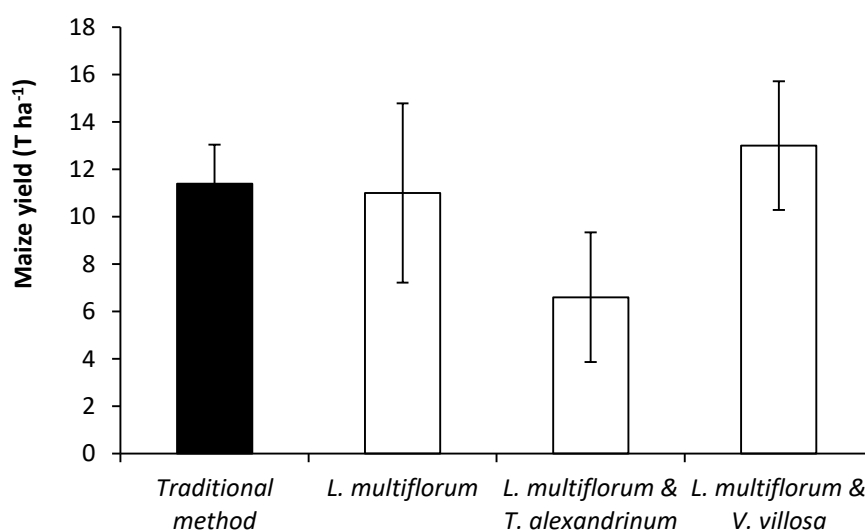


Figure 56 Comparison of maize yield (T ha⁻¹) between the cover crop treatments (white) and the traditional method (black) measured in November 2015. Vertical bars represent SE (n=5). Traditional method= no cover crops, mouldboard plough.

3.3.3 Soil biology

3.3.3.1 Microbial Biomass

There was no a substantial difference in MB between the traditional method and the cover crop treatments (Figure 57). Microbial biomass was not significantly affected by any treatment ($P>0.05$; Figure 57) and location (area A, B and C) ($P>0.05$). However, a 20% reduction in MB was found between November 2015 and March 2016 ($P<0.001$; Figure 58).

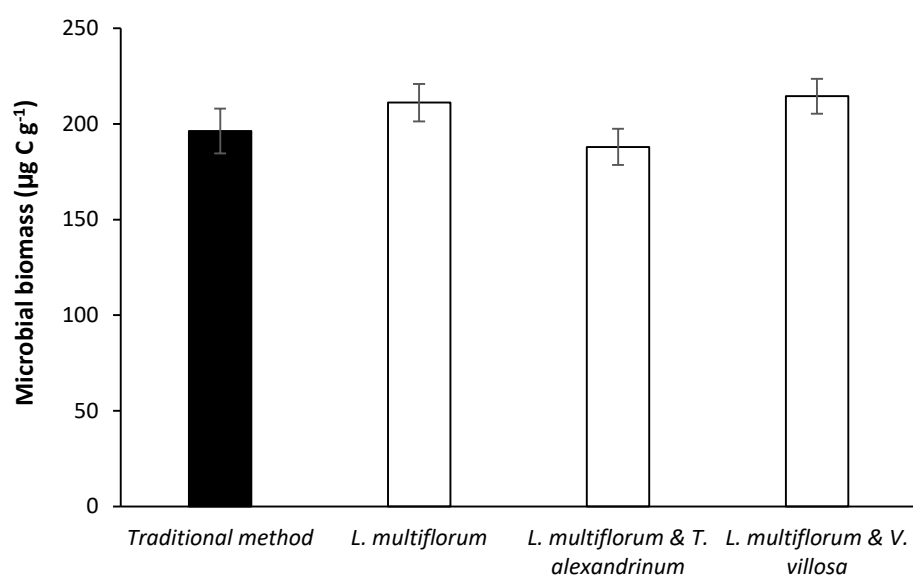


Figure 57 Comparison of microbial biomass ($\mu\text{g C g}^{-1}$) between the cover crop treatments (white) and the traditional method (black) method measured outside the wheel tracks, two sampling points, November 2015 and March 2016. Vertical bars represent SE ($n=10$). Traditional method= no cover crops, mouldboard plough.

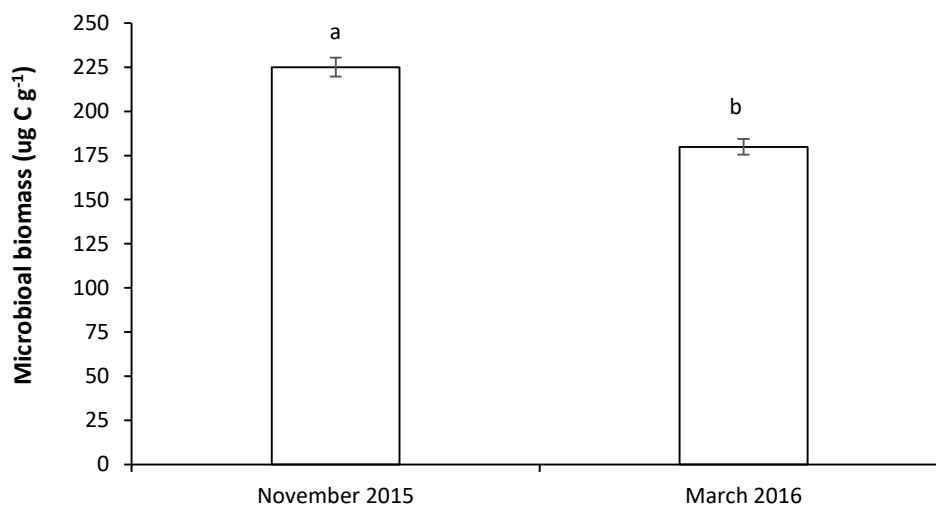


Figure 58 Comparison of microbial biomass ($\mu\text{g C g}^{-1}$) between November 2015 and March 2016 measured outside the wheel tracks. Vertical bars represent SE ($n=20$), letters represent homogenous groupings.

3.3.3.1.1 Microbial biomass: effect of the wheel tracks

Imposed treatments did not affect MB measured in the wheel tracks ($P>0.05$; Figure 59) and no difference was found between the traditional method and the cover crop treatments. Additionally MB measured in the wheel tracks was not significantly different from the MB measured outside the wheel tracks ($P>0.05$; Figure 60).

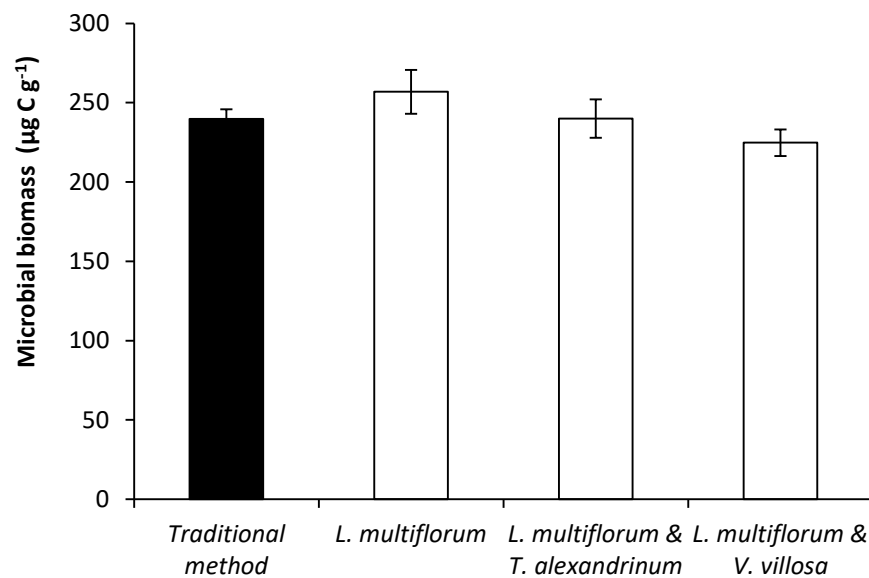


Figure 59 Comparison of microbial biomass ($\mu\text{g C g}^{-1}$) the cover crop treatments (white) and the traditional method (black) measured in the wheel tracks in November 2015. Vertical bars represent SE ($n=5$). Traditional method= no cover crops, mouldboard plough.

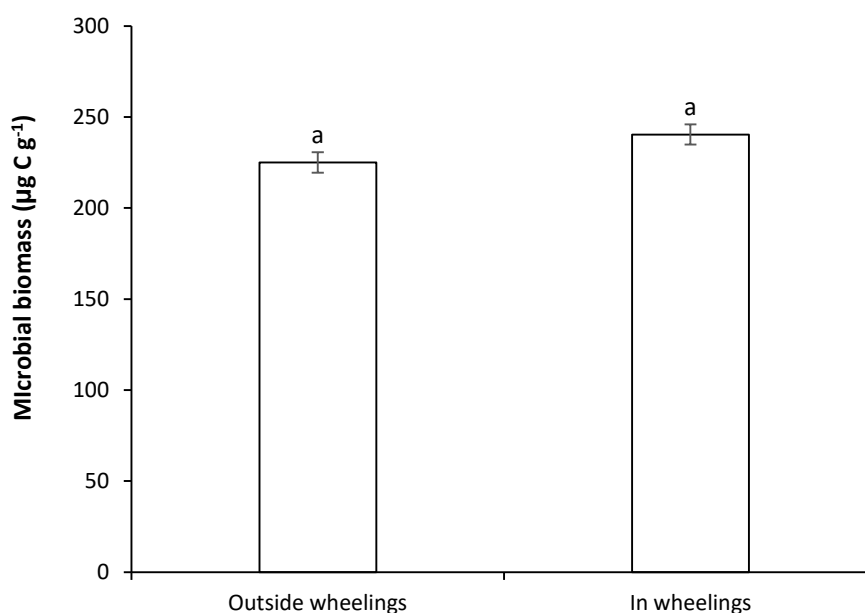


Figure 60 Comparison of microbial biomass ($\mu\text{g C g}^{-1}$) between samples collected outside the wheel tracks and inside the wheel tracks in November 2015. Vertical bars represent SE (n=20), letters represent homogenous groupings.

3.3.3.2 Phospholipid fatty acid analysis

PCA was performed on the mol% of the different microbial phospholipids measured during Experiment 2 (two sampling points November 2015 and March 2016). Principal component 1 (PC 1), PC 2, and PC 3 of the accounted for 59% of the total variation (PC 1 29%, PC 2 19.3%, PC 3 10.4%). On PC1, fatty acids with loadings ≥ 0.8 included 14:0, 15:0i, 15:0ai, 15:0, 16:0i, 16:1 Δ 7c and Me 17:0 isomer; 18:1 Δ 9c had a loading ≤ -0.8 . On PC 2, 17:0 br had a loading ≤ -0.8 .

Significant differences between the treatments were found only on PC 1. *L. multiflorum* & *T. alexandrinum* had a significantly different profile compared to the traditional method, *L. multiflorum* and *L. multiflorum* & *V. Villosa* ($P < 0.05$; Figure 61). However, there was no difference between *L. multiflorum* and *L. multiflorum* & *V. Villosa*. Therefore, there were no distinct differences in the PLFA profile between the traditional method and the cover crop treatments.

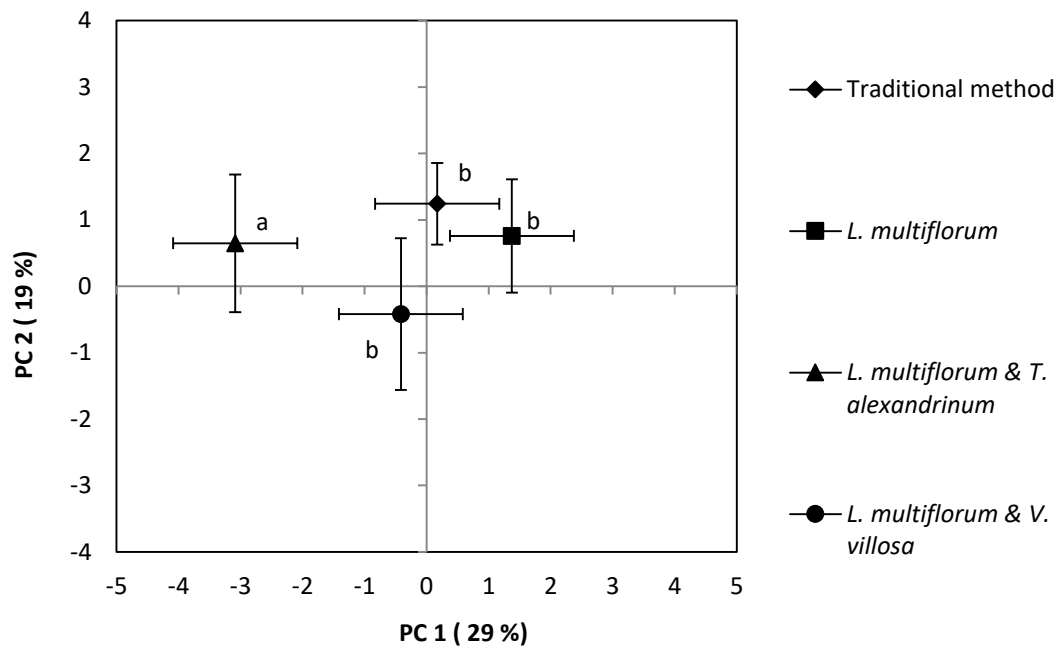


Figure 61 Comparison of microbial community phenotypic profile between the cover crop treatments and the traditional method (two sampling points, November 2015 and March 2016). PCA score plots of the means, PC 1 and 2 ($n=10 \pm SE$), letters represent homogenous groupings on PC 1. Traditional method= no cover crops, mouldboard.

Soil microbial community in March 2016 had a significantly different phenotypic profile on PC 1 compared to November 2015 ($P<0.001$; Figure 62). Additionally, *L. multiflorum* & *T. alexandrinum* in March 2016 had a significantly different profile compared to all the other treatments both in November 2015 and in March 2016 ($P<0.01$; Figure 62), while no significant differences were found between the treatments in November 2015. The traditional method did not move in the score plot from November 2015 to March 2016 as much as the cover crop treatments.

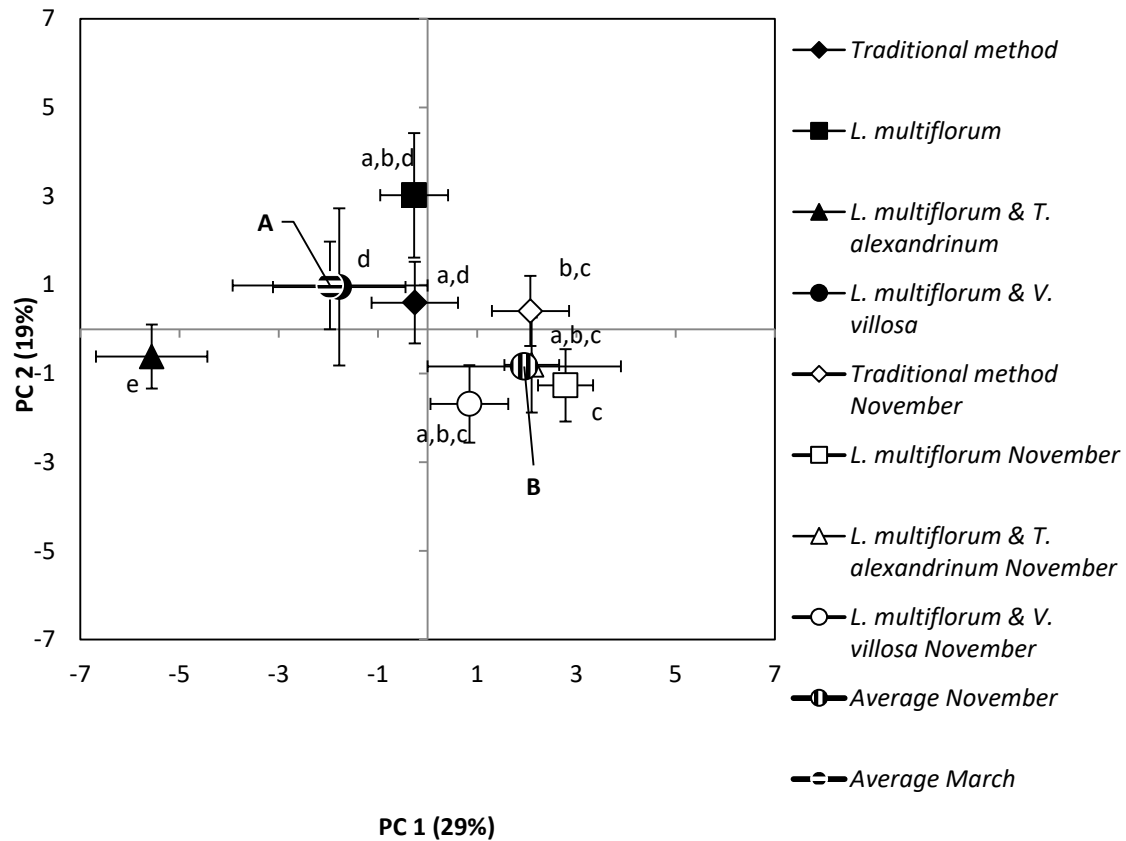


Figure 62 Comparison of microbial community phenotypic profile between the cover crop treatments and the traditional method. PCA score plots of the means, PC 1 and 2 (Treatments $n=5 \pm SE$; Sampling date $n=20$). Capital letters represent homogenous groupings on PC 1 for Sampling date effect. Lower case letters represent homogenous groupings on PC 1 for sampling date x Treatment effect. Traditional method= no cover crops, mouldboard.

3.3.3.1 Phospholipid fatty acid analysis: effect of the wheel tracks

Principal component 1, PC 2, and PC 3 of the PCA performed on the mol% of the different microbial phospholipids measured in the wheel tracks accounted for 65% of the total variation (PC 1 32%, PC 2 18%, PC 3 15%). On PC 1, 15:0i had a loading ≥ 0.8 ; on PC 2 18:1 ω 7c had a loading ≥ 0.8 .

There were no significant differences in the phenotypic profile of the microbial community in the wheel tracks between the cover crop treatments and the

traditional method ($P>0.05$; Figure 63). Additionally, there were no differences within the cover crop treatments ($P>0.05$; Figure 63).

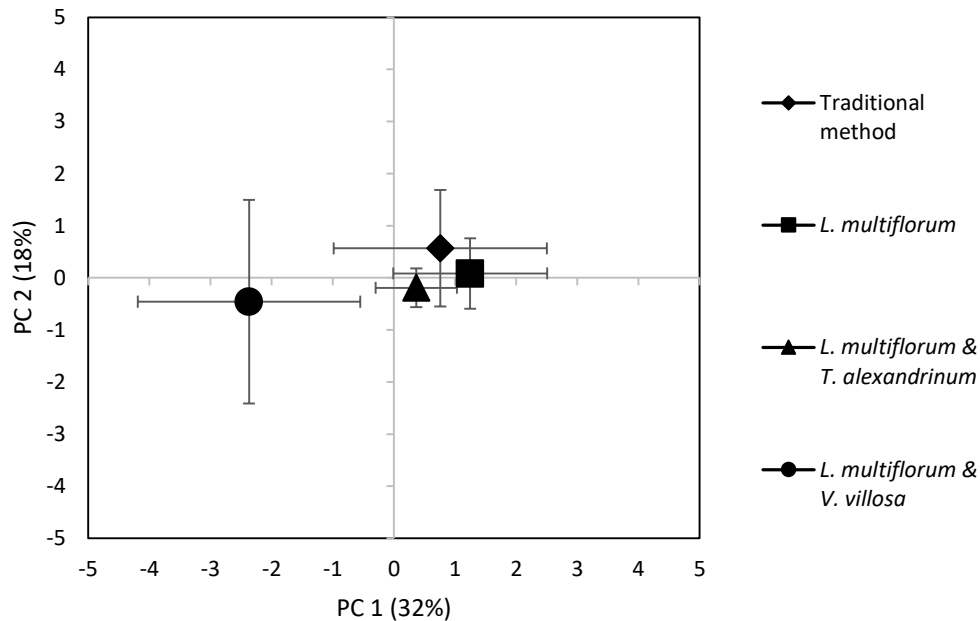


Figure 63 Comparison of microbial community phenotypic profile between the cover crop treatments and the traditional method in the wheel tracks in November 2015. PCA score plots of the means, PC 1 and 2 (Treatments $n=5 \pm$ SE). Traditional method= no cover crops, mouldboard.

Significant differences were found between samples collected in wheel tracks compared to samples collected outside the wheel tracks on PC 1 ($P<0.05$; Figure 54). However, there were no significant differences between the treatments ($P>0.05$; Figure 54). Principal component 1, PC 2, and PC 3 accounted for 57% of the total variation (PC 1 28.8%, PC 2 14.6%, PC 3 14.0%). On PC 1, 15:0i had a loading ≥ 0.8 .

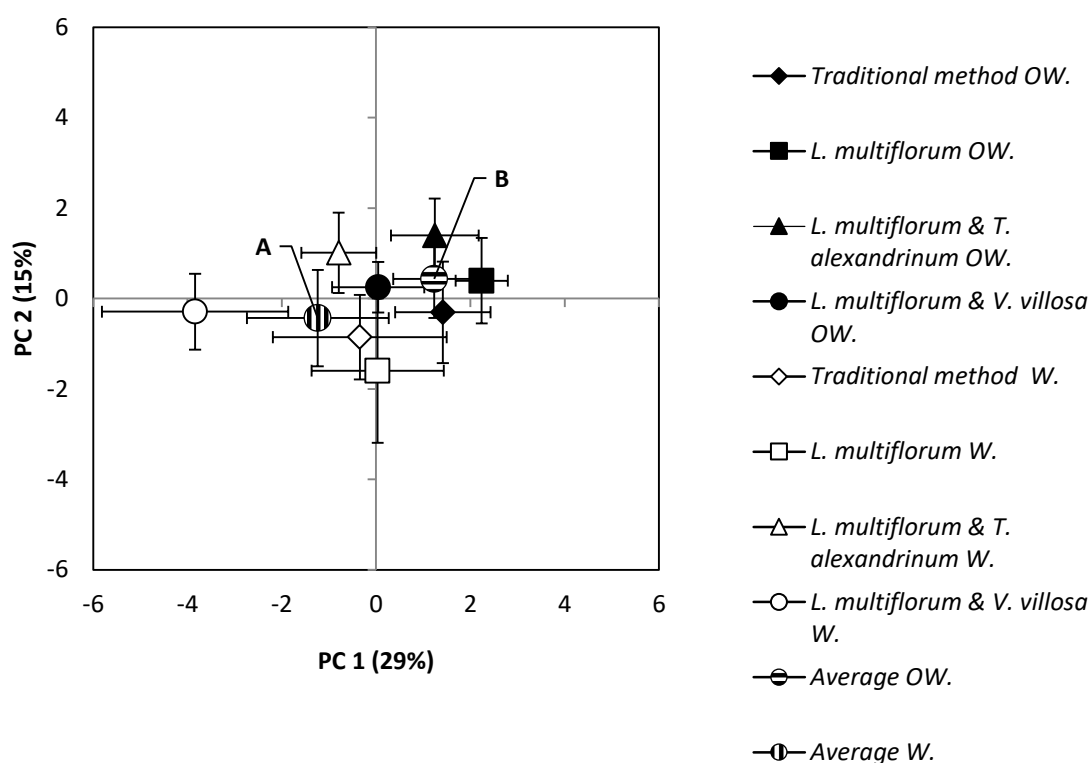


Figure 64 Comparison of microbial community phenotypic profile between the cover crop treatments and the traditional method measured in the wheel tracks (white) and outside the wheel tracks (black). PCA score plots of the means, PC 1 and 2 (Treatments $n=5 \pm SE$; Wheel tracks $n=20$). Letters represent homogenous groupings on PC 1. Traditional method= no cover crops, mouldboard. OW. = outside the wheel tracks.

3.3.3.2 Fatty acid indicators

Treatments and sampling date affected all fatty acid indicators (total bacteria, total fungi, Gram-positive bacteria, Gram-negative bacteria, Fungi/Bacteria, Arbuscular mycorrhizae), while location significantly affected only Gram-negative bacteria (Table 18). The combined effect of treatment and sampling date significantly affected Total fungi, Gram-negative bacteria and Fungi/Bacteria.

Table 18 Summary of the ANOVA P values of the relative abundance of fungi and bacteria.

P				
Fatty acid Indicator (Relative abundance)	Treatment	Sampling date	Treatment x Sampling date	Location
Bacteria	<0.01	<0.001	>0.05	>0.05
Fungi	<0.01	<0.001	<0.01	>0.05
Gram positive bacteria	<0.01	<0.001	>0.05	>0.05
Gram negative bacteria	<0.05	<0.001	<0.05	<0.01
Fungi/Bacteria	<0.01	<0.05	<0.01	>0.05
Arbuscular mycorrhizae	<0.05	>0.05	<0.05	<0.01

3.3.3.2.1 Relative abundance of bacteria

There was a significant difference in the relative abundance of bacteria between the traditional method and the cover crop mixtures. The traditional method had 7% larger relative abundance of bacteria compared to *L. multiflorum* & *T. alexandrinum* and 8% larger compared to *L. multiflorum* & *V. villosa* ($P < 0.01$; Figure 65). However, there were no differences between *L. multiflorum* and the traditional method.

Considering the cover crop treatments, *L. multiflorum* had 6% larger relative abundance of bacteria compared to *L. multiflorum* & *T. alexandrinum* and 7% larger compared to *L. multiflorum* & *V. villosa*. Moreover, there were no differences between *L. multiflorum* & *T. alexandrinum* and *L. multiflorum* & *V. villosa*.

There was a 12% reduction of in relative abundance of bacteria from November 2015 to March 2016 ($P<0.001$; Figure 66). Although, there was no significant interaction between sampling date and treatment ($P>0.05$) and location did not affect treatments ($P>0.05$).

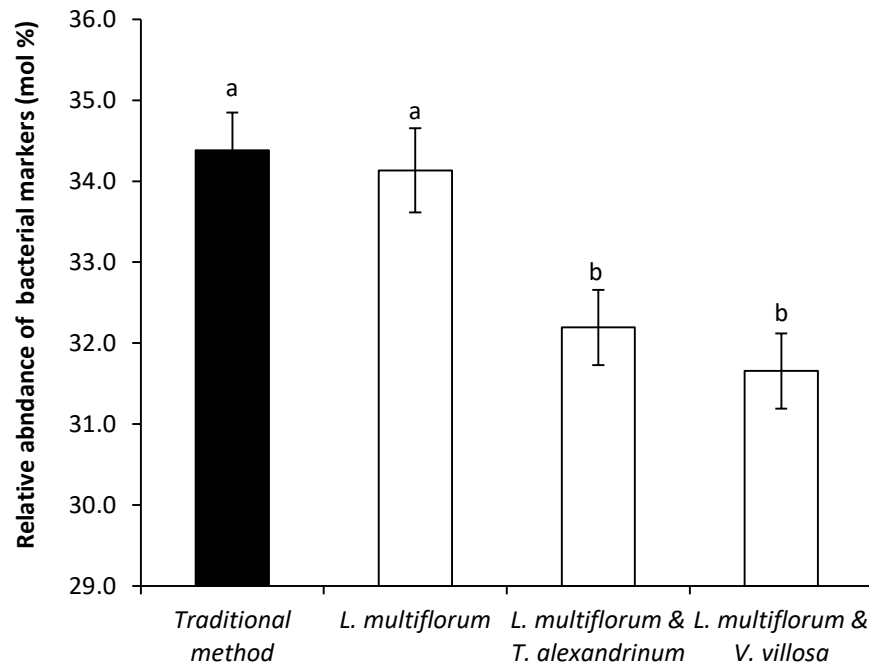


Figure 65 Comparison of relative abundance of bacteria (mol %) measured between the cover crop treatments (white) and the traditional method (black) (two sampling points, November 2015 and March 2016). Vertical bars represent SE (n=10), letters represent homogenous groupings. The traditional method= no cover crops, mouldboard.

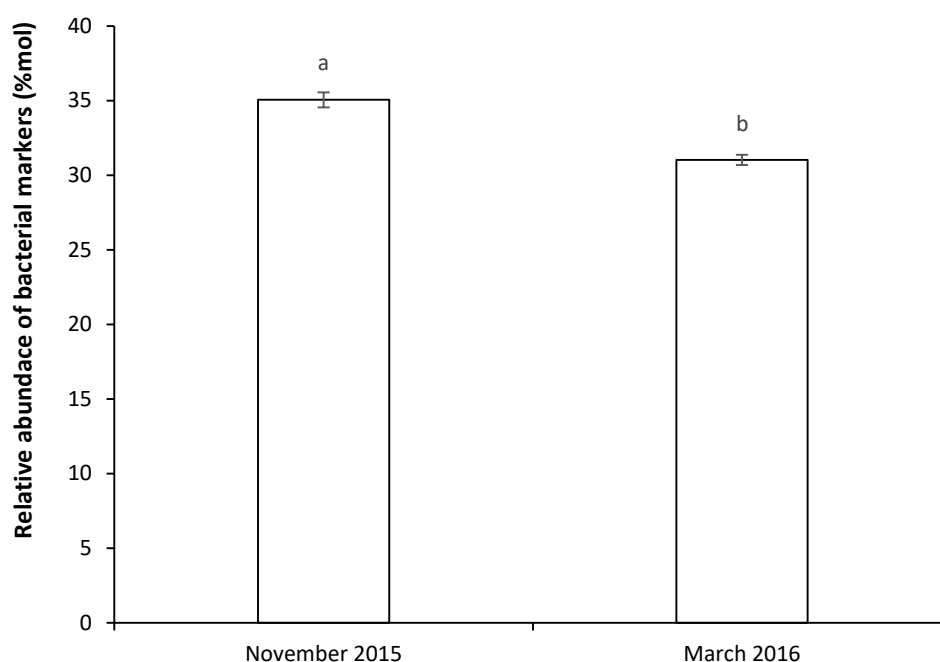


Figure 66 Comparison of relative abundance of bacteria (mol %) (two sampling points, November 2015 and March 2016). Vertical bars represent SE (n=10), letters represent homogenous groupings.

3.3.3.2.1.1 Relative abundance of Gram-positive bacteria

Significant differences were found between the treatments ($P < 0.01$; Figure 67). *L. multiflorum* & *V. villosa* had 10% lower relative abundance of Gram-positive bacteria compared to the traditional method, 7% lower compared to *L. multiflorum* and 6% lower compared to *L. multiflorum* & *T. alexandrinum* (Figure 67). However, there was not a distinct difference in relative abundance of Gram-positive bacteria between the traditional method and the cover crop treatments. As, no differences were found between the traditional method, *L. multiflorum* and *L. multiflorum* & *T. alexandrinum*.

In March 2016, the relative abundance of Gram-positive bacteria was 11% lower than in November ($P < 0.001$; Figure 68). However, there was not a significant interaction between sampling date and treatment ($P > 0.05$) and location did not affect treatments ($P > 0.05$).

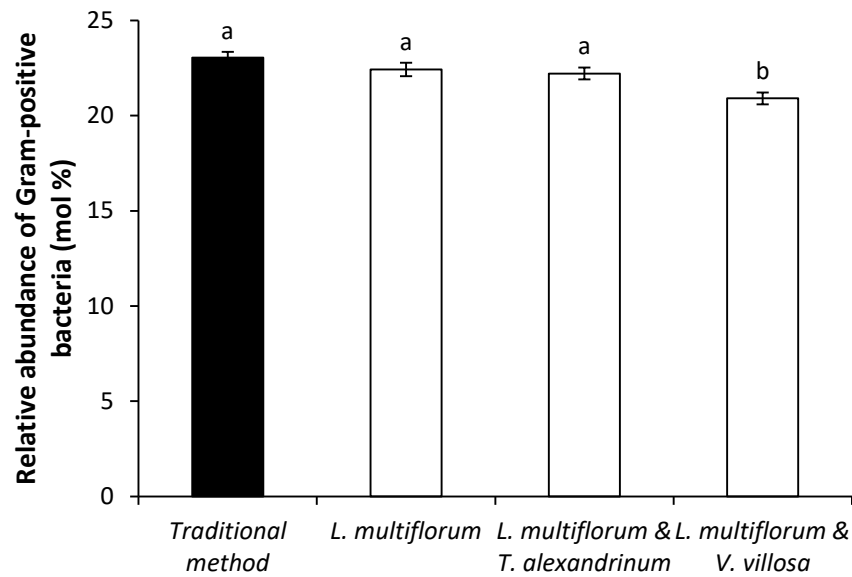


Figure 67 Comparison of relative abundance of Gram-positive bacteria (mol %) between cover crop treatments (white) and the traditional method (black), two sampling points, November 2015 and March 2016. Vertical bars represent SE (n=10), letters represent homogenous groupings. The traditional method= no cover crops, mouldboard.

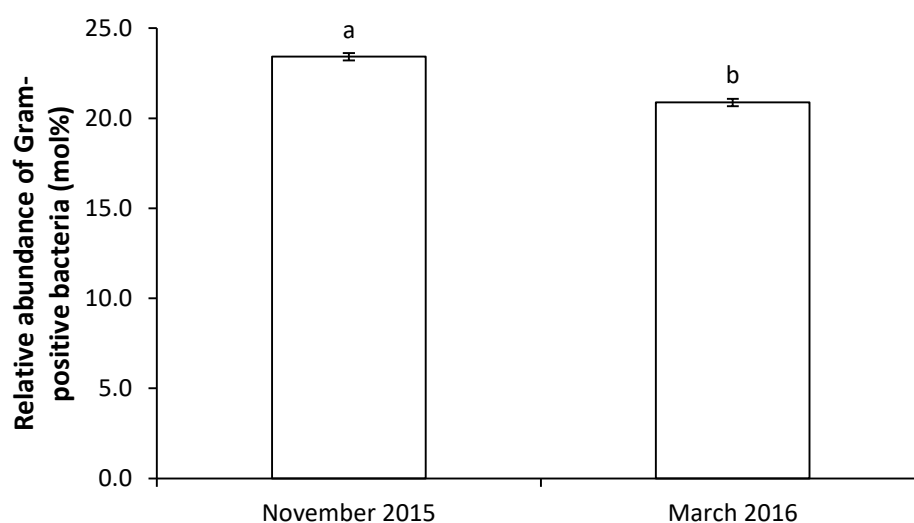


Figure 68 Comparison of relative abundance of Gram-positive bacteria (mol %) between November 2015 and March 2016. Vertical bars represent SE (n=20), letters represent homogenous groupings.

3.3.3.2.1.2 Relative abundance of Gram-negative bacteria

Significant differences were found between the treatments ($P < 0.05$; Figure 69). *L. multiflorum* & *T. alexandrinum* had 14% lower relative abundance of Gram-negative bacteria compared to the traditional method and 18% lower compared to *L. multiflorum* (Figure 69). Yet, *L. multiflorum* & *T. alexandrinum* was not significantly different from *L. multiflorum* & *V. villosa*. Moreover, no differences were found between the traditional method, *L. multiflorum* and *L. multiflorum* & *V. villosa*. Therefore, there was no distinct difference in the relative abundance of Gram-negative bacteria between the traditional method and the cover crop treatments (Figure 69).

In March 2016, the relative abundance of Gram-negative bacteria was 14% lower than in November 2015 ($P < 0.001$; Figure 70). However, there was not a significant interaction between sampling date and treatment ($P > 0.05$). There were significant differences between locations ($P < 0.01$; Figure 71). Location A had 17% lower relative abundance of Gram-negative bacteria compared to B.

Location C had 11% lower abundance compared to B. However, there were no differences between A and C.

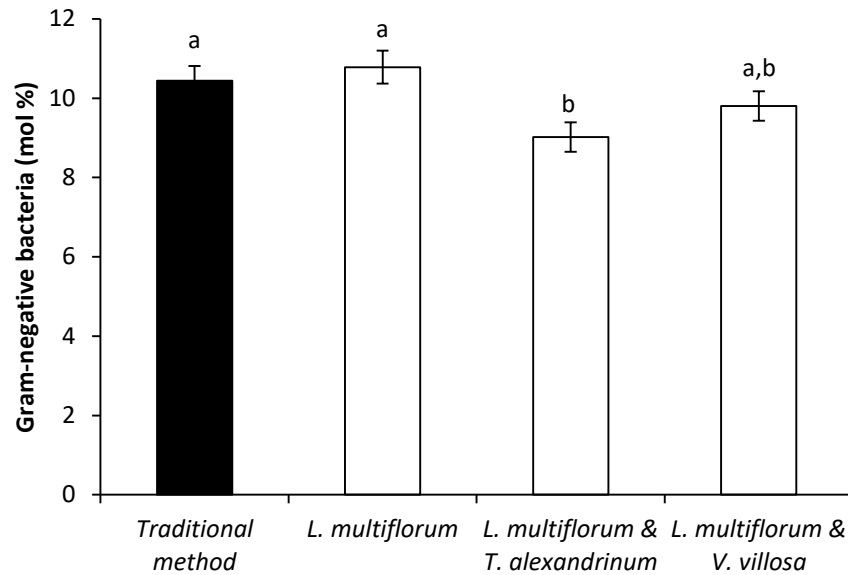


Figure 69 Comparison of the relative abundance of Gram-negative bacteria (mol %) between cover crop treatments (white) and the traditional method (black), two sampling points, November 2015 and March 2016. Vertical bars represent SE (n=10), letters represent homogenous groupings. The traditional method= no cover crops, mouldboard.

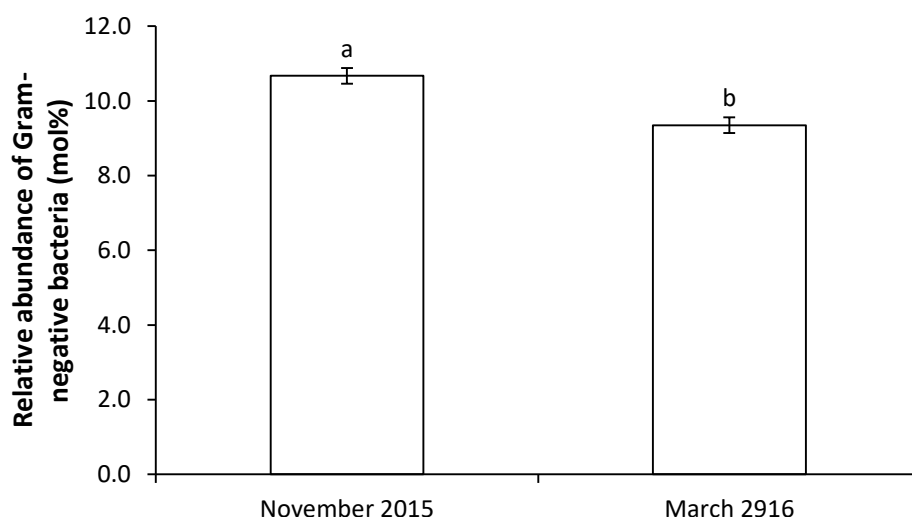


Figure 70 Comparison of the relative abundance of Gram-negative bacteria (mol %) between November 2015 and March 2016. Vertical bars represent SE (n=20), letters represent homogenous groupings.

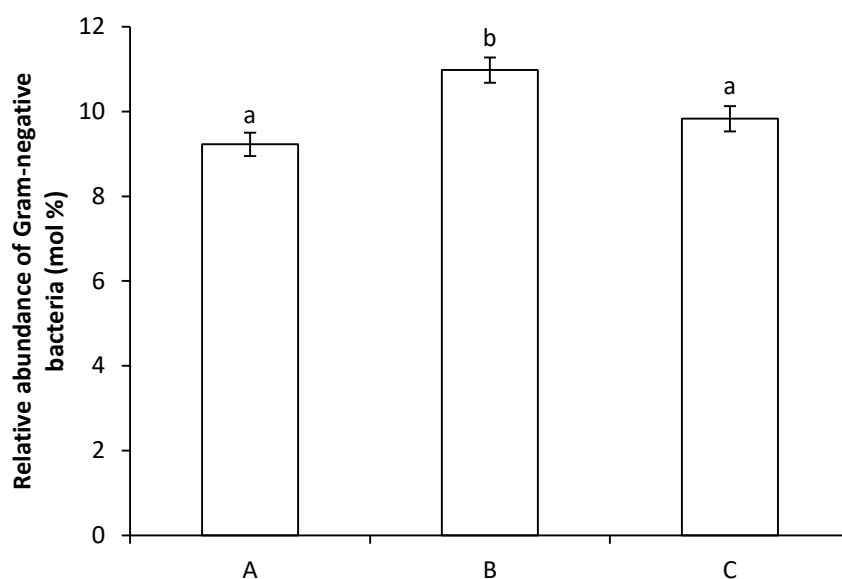


Figure 71 Comparison of relative abundance of Gram-negative bacteria (mol %) between locations, two sampling points, November 2015 and March 2016. Vertical bars represent SE (A n=16, B n=12, C n=12). Lower case letters represent homogenous groupings. . A= west plots; B= central plots (depression); C= east plots.

3.3.3.2.2 Relative abundance of fungi

There was a significant difference in relative abundance of fungi between the traditional method and the cover crop treatments ($P < 0.05$; Figure 72). The traditional method had 31% less relative abundance of fungi compared to *L. multiflorum*, 24% lower compared to *L. multiflorum* & *T. alexandrinum* and 35% lower compared to *L. multiflorum* & *V. villosa*. *L. multiflorum* & *V. villosa* had 12.5% larger fungal marker compared to *L. multiflorum* and 15% larger compared to *L. multiflorum* & *T. alexandrinum*.

There were no differences within the cover crop treatments, as there were no differences between *L. multiflorum* & *T. alexandrinum*, and *L. multiflorum*, and between *L. multiflorum* and *L. multiflorum* & *V. villosa*. There was a 46% increase in fungal marker relative abundance from November 2015 to March 2016 ($P < 0.001$; Figure 73).

Additionally, the interaction between treatment and sampling date had a significant effect ($P < 0.01$; Figure 74). There were no significant differences between the samples collected in November 2015 and the traditional method in March 2016. Yet, in regards of the samples collected in November 2015, all treatments had on average 71% lower relative abundance of fungi compared to *L. multiflorum* in March 2016, 69% compared to *L. multiflorum* & *T. alexandrinum* in March 2016 and 83% compared to *L. multiflorum* & *V. villosa* in March 2016. In contrast, location did not have a significant effect ($P > 0.05$).

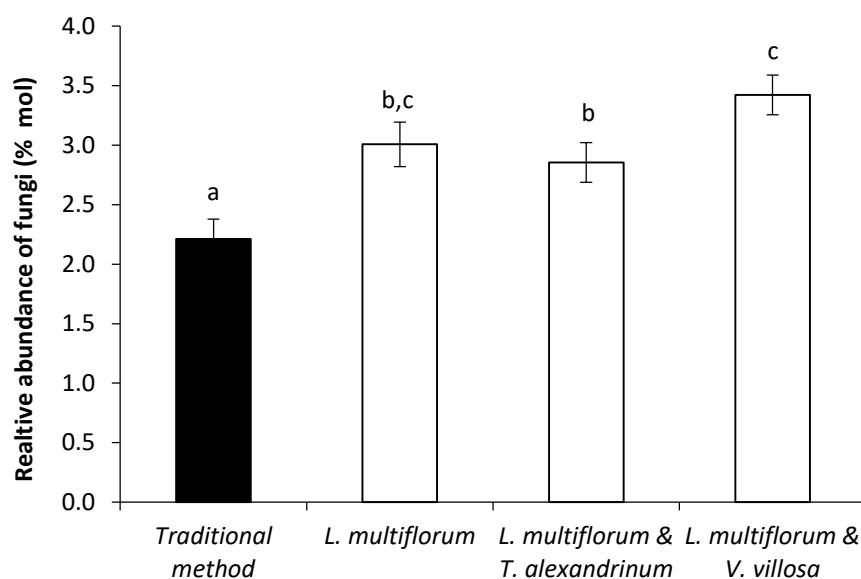


Figure 72 Comparison of relative abundance of fungi (mol %) between cover crop treatments (white) and the traditional method (black), two sampling points, November 2015 and March 2016. Vertical bars represent SE (n=10), letters represent homogenous groupings. The traditional method= no cover crops, mouldboard.

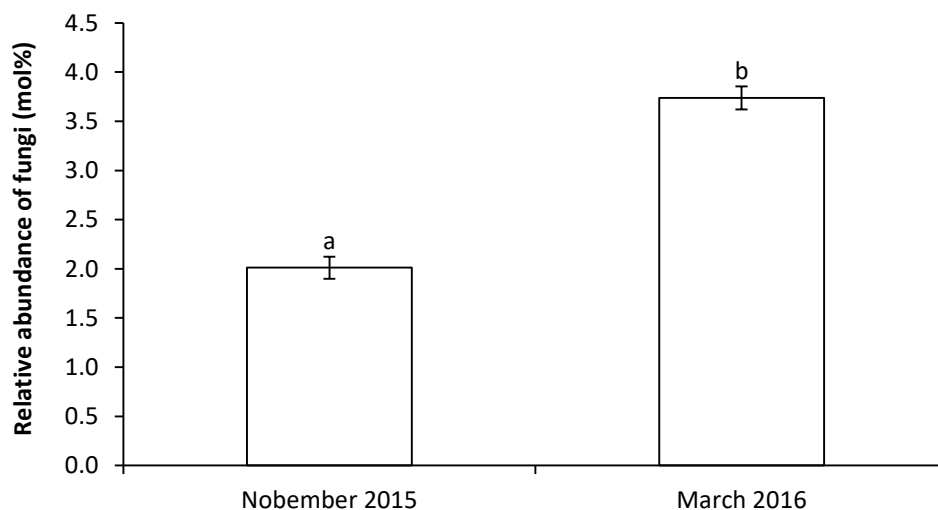


Figure 73 Comparison of relative abundance of fungi (mol %) between November 2015 and March 2016. SE (n=20), letters represent homogenous groupings.

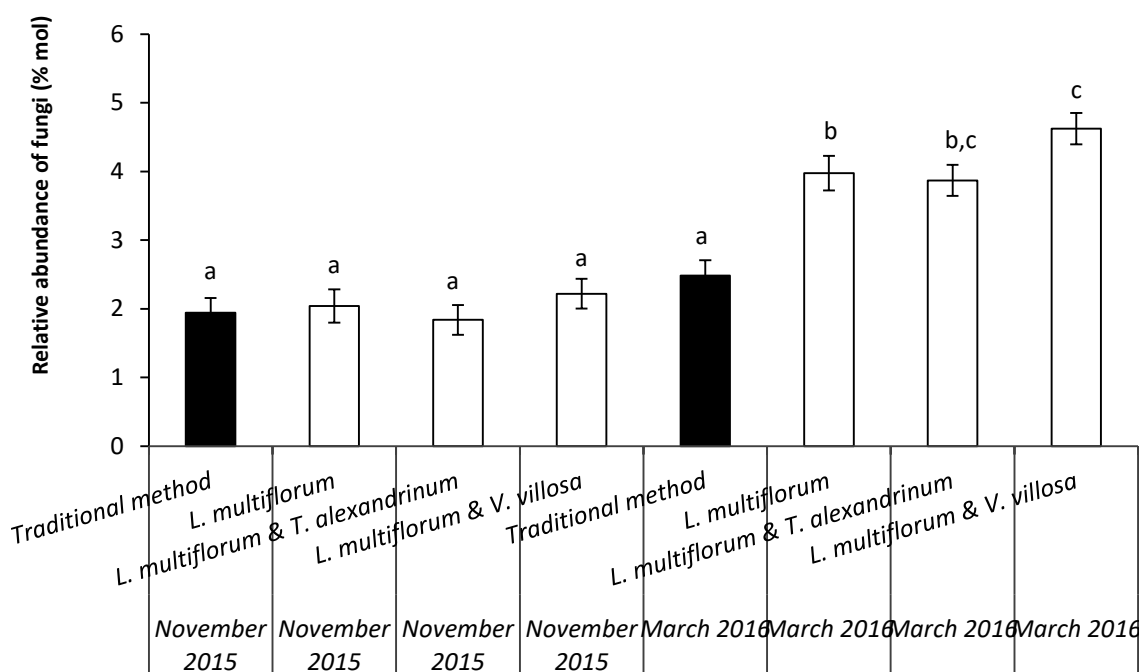


Figure 74 Comparison of relative abundance of fungi (mol %) between cover crop treatments (white) and the traditional method (black), two sampling points, November 2015 and March 2016. The traditional method= no cover crops, mouldboard. Vertical bars represent SE (n=5), letters represent homogenous groupings.

3.3.3.3 Ratio between fungi and bacteria

There was a significant difference in F/B between the traditional method and the cover crop treatments ($P < 0.001$; Figure 75). The traditional method had 59% lower F/B compared to *L. multiflorum* & *V. villosa* and 40% lower compared to *L. multiflorum* and compared *L. multiflorum* & *T. alexandrinum*.

In reference to the cover crop treatments, *L. multiflorum* & *V. villosa* had 20% lower F/B compared to *L. multiflorum* & *T. alexandrinum* and compared to *L. multiflorum*. Yet, there were no differences between *L. multiflorum* & *T. alexandrinum* and *L. multiflorum*. Significant differences were found between sampling date ($P < 0.05$; Figure 76). There was a 67% increase in the F/B from

November 2015 to March 2016. However, location did not have a significant effect ($P>0.05$).

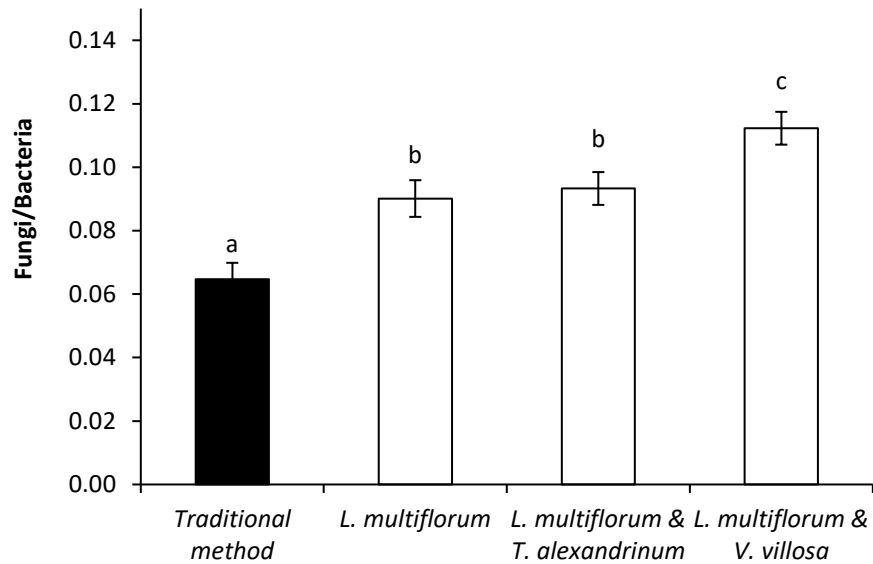


Figure 75 Comparison of F/B between cover crop treatments (white) and the traditional method (black), two sampling points, November 2015 and March 2016. Vertical bars represent SE (n=10), letters represent homogenous groupings. The traditional method= no cover crops, mouldboard.

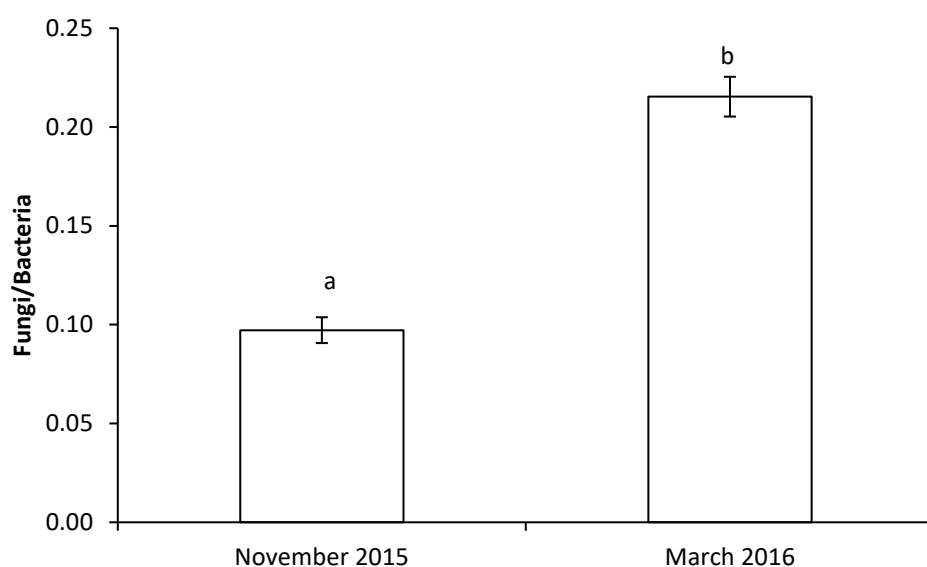


Figure 76 Comparison of F/B between November 2015 and March 2016. SE (n=20), letters represent homogenous groupings.

The interaction between treatment and sampling date also affected F/B ($P < 0.01$; Figure 77). There were no significant differences in F/B between the samples collected in November 2015. Although, on average, treatments in November had 68% lower F/B compared to *L. multiflorum*, 77% compared to *L. multiflorum* & *T. alexandrinum* and 90% compared to *L. multiflorum* & *V. villosa* in March 2016.

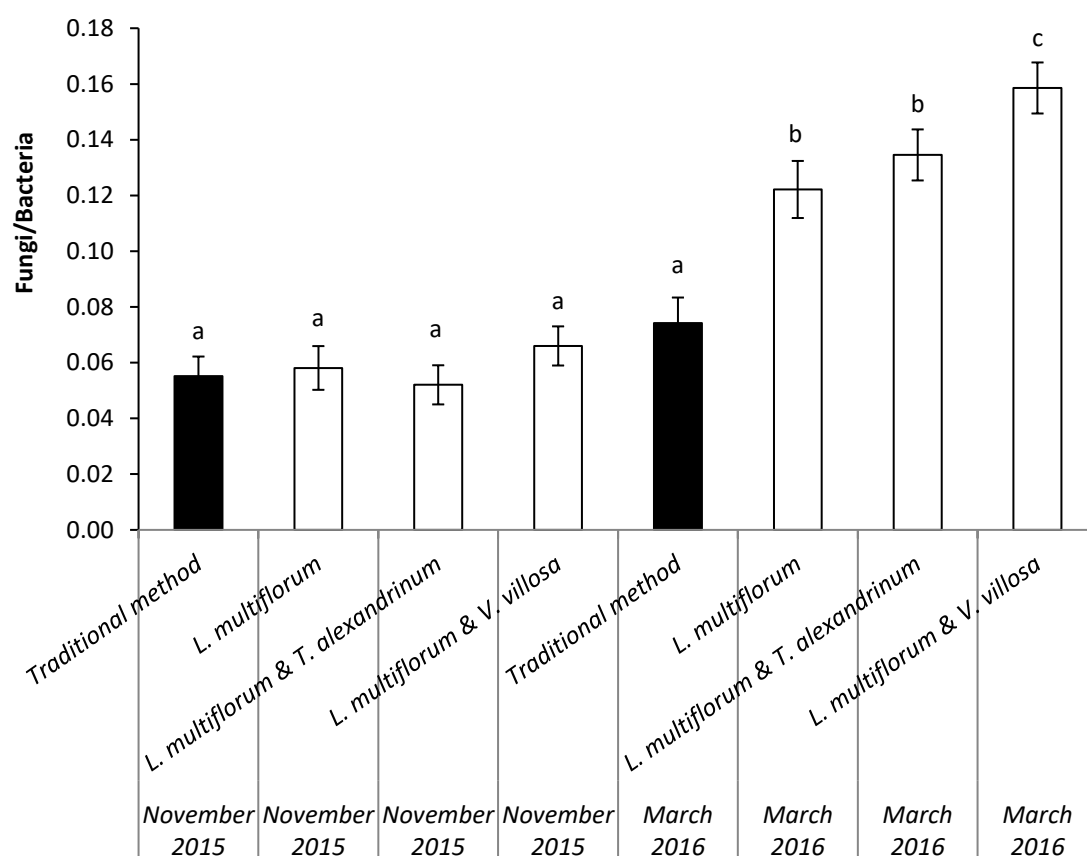


Figure 77 Comparison of F/B between cover crop treatments (white) and the traditional method (black), two sampling points, November 2015 and March 2016. The traditional method= no cover crops, mouldboard. Vertical bars represent SE (n=5), letters represent homogenous groupings.

3.3.3.3.1 Relative abundance of arbuscular mycorrhizae

Significant differences in relative abundance of AMF were found between the treatments ($P < 0.05$; Figure 78). *L. multiflorum* & *T. alexandrinum* had 17% lower relative abundance of AMF compared to the traditional method and compared to *L. multiflorum*. However, there were no difference between *L. multiflorum* & *T. alexandrinum* and *L. multiflorum* & *V. villosa*, and between *L. multiflorum* and the traditional method. Therefore, there was no clear difference in the relative abundance of arbuscular mycorrhizae between the traditional method and the cover crop treatments (Figure 78).

Sampling date did not have a significant effect ($P>0.05$). Nevertheless, there was a significant interaction between sampling date and treatment ($P<0.05$; Figure 79). There were no significant differences between the traditional methods (November 2015 and March 2016), *L. multiflorum* in March, *L. multiflorum* & *T. alexandrinum* in November 2015 and *L. multiflorum* & *V. villosa* treatments (November 2015 and March 2016). *L. multiflorum* & *T. alexandrinum* in March 2016 was significantly lower compared to the rest of the treatments, 18% lower compared to the average. There were significant differences between locations ($P<0.01$; Figure 80). Plots in location A and C had 20% lower relative abundance of AMF compared to location B (depression). There were no differences between A and C.

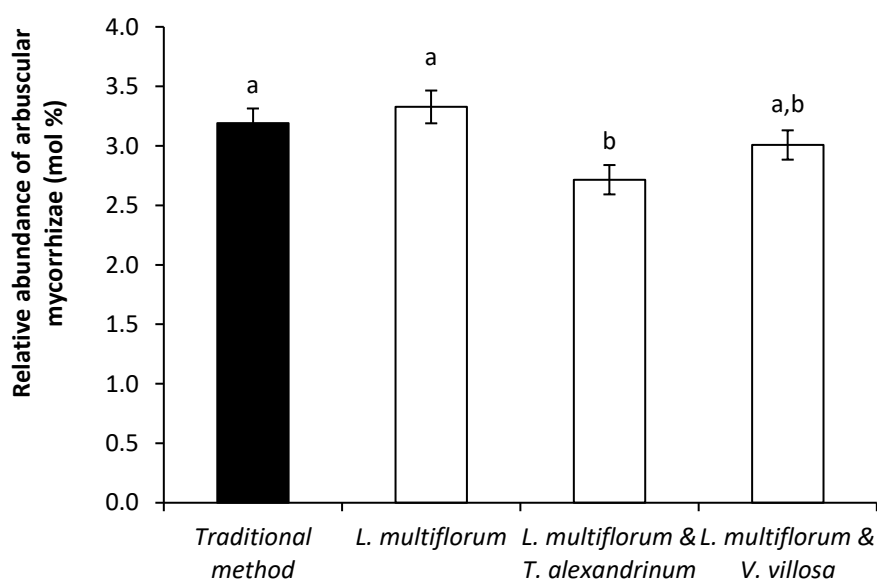


Figure 78 Comparison of relative abundance of arbuscular mycorrhizae (mol %) between cover crop treatments (white) and the traditional method (black), two sampling points, November 2015 and March 2016. Vertical bars represent SE (n=10), letters represent homogenous groupings. The traditional method= no cover crops, mouldboard.

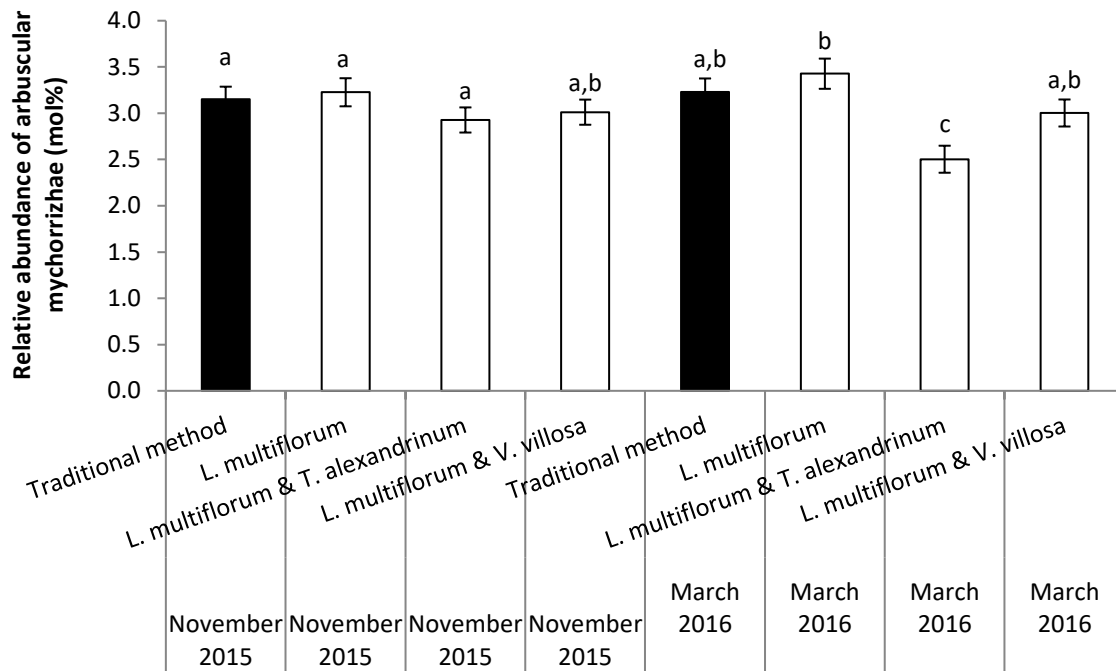


Figure 79 Comparison of relative abundance of arbuscular mycorrhizae (mol %) between cover crop treatments (white) and the traditional method (black) in November 2015 and March 2016. The traditional method= no cover crops, mouldboard. Vertical bars represent SE (n=5), letters represent homogenous groupings.

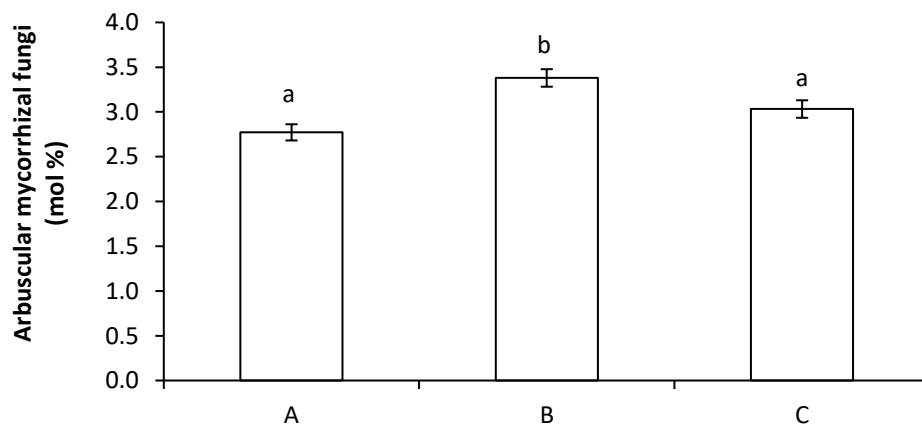


Figure 80 Comparison of relative abundance of arbuscular mycorrhizal fungi (mol %) between locations (two sampling points, November 2015 and March 2016). Vertical bars represent SE (A n=16, B n=12, C n=12). Letters represent homogenous groupings. A= west plots; B= central plots (depression); C= east plots.

3.3.3.4 Multiple substrates induced respiration

PCA was performed on the multiple substrate respiration rates ($\mu\text{g CO}_2 \text{ g}_{\text{DWsoil}}^{-1} \text{ h}^{-1}$) measured during Experiment 2 (two sampling points November 2015 and March 2016). Principal component 1, PC 2 and PC 3 accounted for 70% of the total variation (PC 1 28.3%, PC 2 25.8% and PC 3 19.3%). Substrates with loadings ≥ 0.79 included arginine on PC 1 and citric acid on PC 2. Imposed treatments did not affect PCs ($P>0.05$; Figure 81) and there was no clear difference between the traditional method and the cover crop treatments. However, the functional profile was significantly different between November 2015 and March 2016 ($P<0.01$; Figure 81).

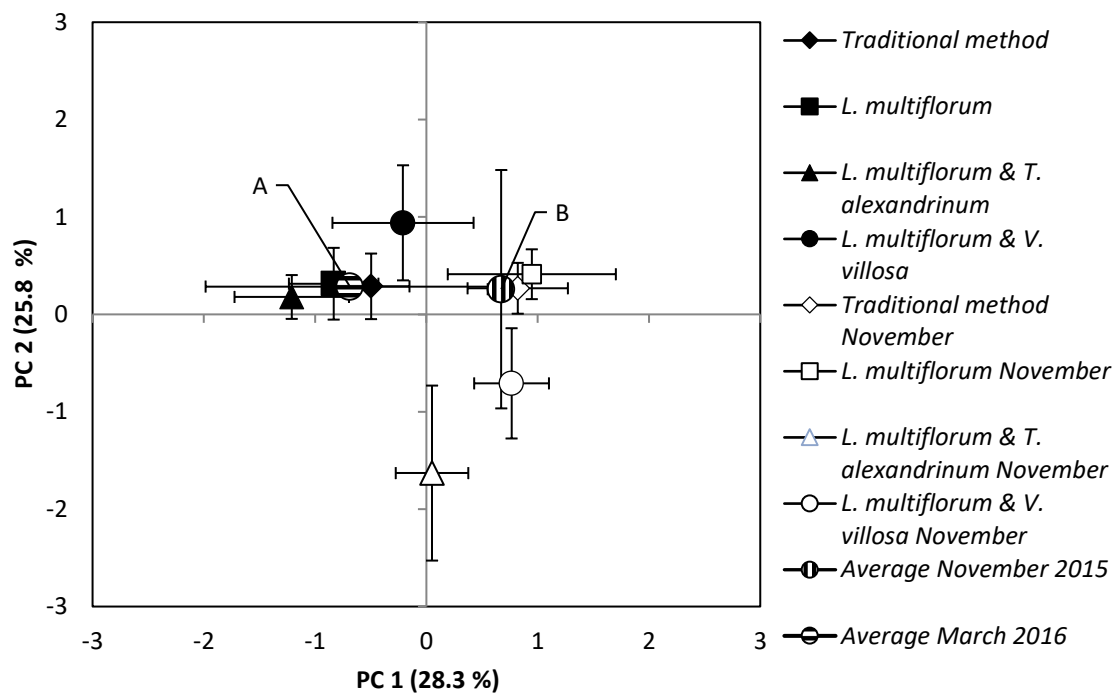


Figure 81 Comparison of microbial community functional profile between the cover crop treatments and the traditional method. PCA score plots of the means, PC 1 and 2 (Treatments $n=5 \pm \text{SE}$; Sampling date $n=20 \pm \text{SE}$). Samples collected in November 2015 (white) and samples collected in March 2016 (black). Letters represent homogenous groupings on PC 1 (Sampling date). Traditional method= no cover crops, mouldboard.

3.3.3.1 Multiple substrates induced respiration: effect of the wheel tracks

Principal component 1, PC 2, and PC 3 of the PCA performed on the multiple substrate respiration rates ($\mu\text{g CO}_2 \text{ g}_{\text{DWsoil}}^{-1} \text{ h}^{-1}$) measured in the wheel tracks accounted for 71% of the total variation (PC 1 27.7%, PC 2 25.6%, PC 3 17.4%). Glucose had a loading ≥ 0.7 on PC 1; ketoglutaric acid had a loading ≤ -0.8 on PC 2.

Imposed treatments did not affect the functional profile ($P > 0.05$; Figure 82). There were no differences between traditional method and the cover crop treatments (Figure 82). Additionally, there were no differences within the cover crop treatments.

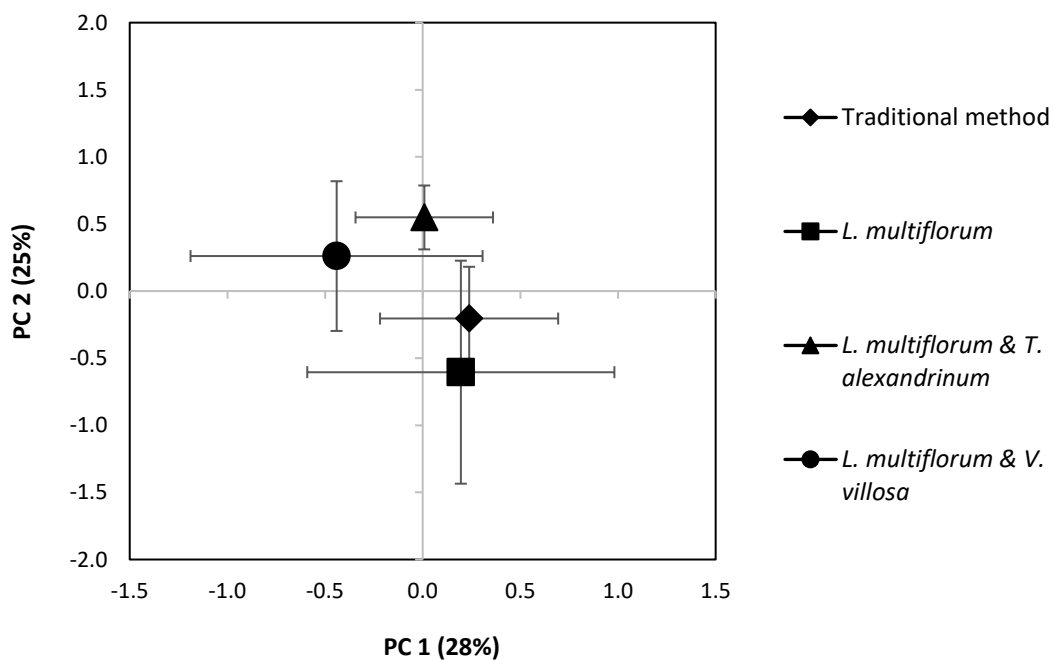


Figure 82 Comparison of the microbial community functional profile between the cover crop treatments and the traditional method measured in the wheel tracks in November 2015. PCA score plots of the means, PC 1 and 2 (Treatments $n=5 \pm \text{SE}$). Traditional method= no cover crops, mouldboard.

Principal component 1, PC 2 and PC 3 of the PCA performed on the multiple substrate respiration rates ($\mu\text{g CO}_2 \text{ g}_{\text{DWsoil}}^{-1} \text{ h}^{-1}$) measured in the wheel tracks and outside the wheel tracks accounted for 67% of the total variation (PC 1 26.5%, PC 2 22.7% and PC 3 17.1%). Substrates with loadings ≤ -0.78 included glucose on PC 1 and glutamine on PC 2. There were no differences in the functional profile of the samples collected in the wheel tracks (white) compared to the samples collected outside the wheel tracks (black; $P > 0.05$; Figure 83).

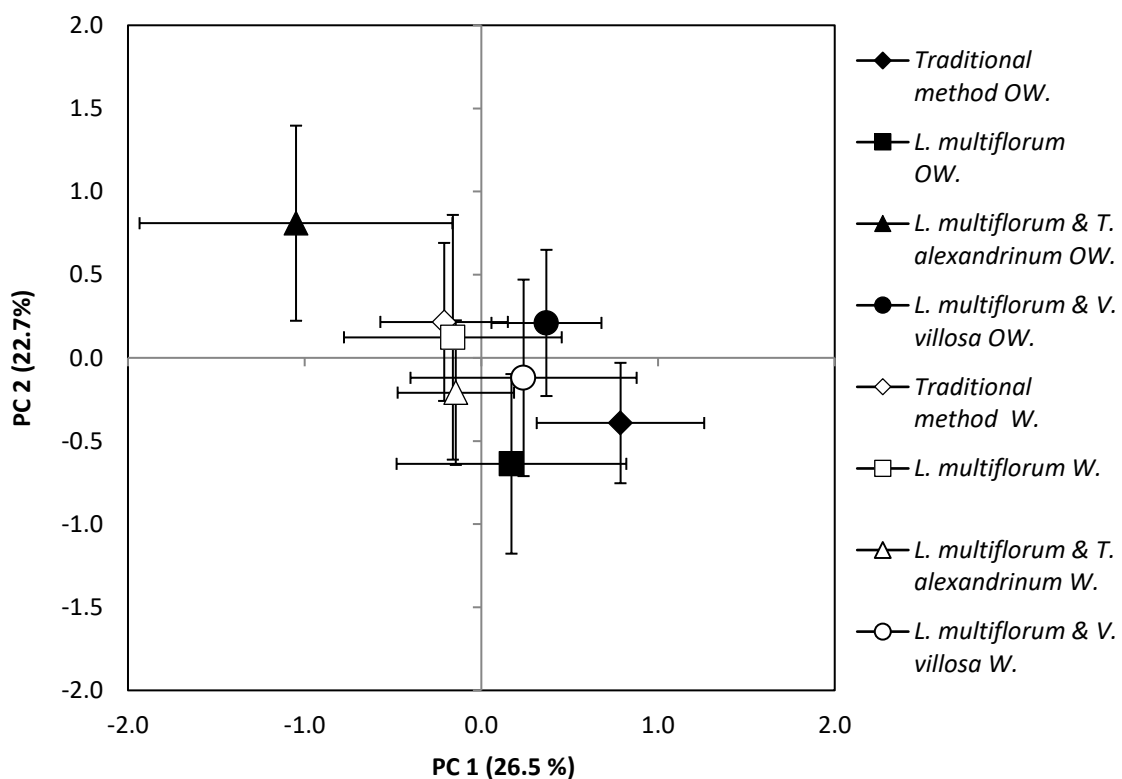


Figure 83 Comparison of microbial community functional profile between the cover crop treatments and the traditional method in the wheel tracks (white) and outside the wheel tracks (black). PCA score plots of the means, PC 1 and 2 (Treatments $n=5 \pm \text{SE}$; Wheel tracks $n=20$). Letters represent homogenous groupings on PC 1. Traditional method= no cover crops, mouldboard. OW. = outside the wheel tracks.

3.3.3.2 Basal and glucose respiration rate

The traditional method did not have different basal and glucose respiration rates compared to the cover crop treatments (Figure 84 and 85). Additionally, there were no significant differences between any of the treatments ($P>0.05$) and sampling date did not have a significant effect ($P>0.05$).

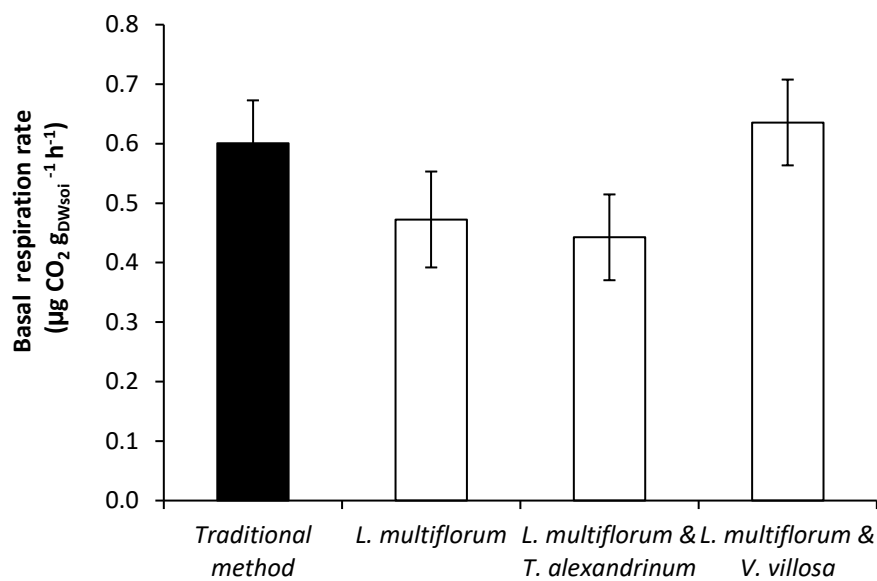


Figure 84 Comparison of basal respiration rates between cover crop treatments (white) and the traditional method (black) measured between November 2015 and March 2016. The traditional method= no cover crops, mouldboard. Vertical bars represent SE (n=10).

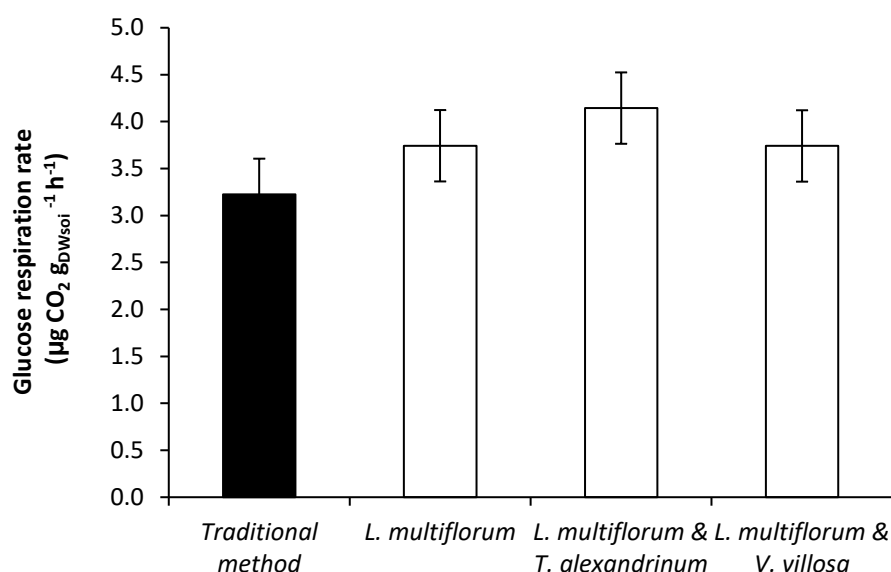


Figure 85 Comparison of glucose respiration rates between cover crop treatments (white) and the traditional method (black) measured between November 2015 and March 2016. The traditional method= no cover crops, mouldboard. Vertical bars represent SE (n=10).

3.3.3.3 Fungal biomass and fungal/bacterial biomass ratio

The average recovery for fungal biomass (ergosterol) analysis was 86%. No significant difference was found between treatments ($P > 0.05$; Figure 86). Additionally, fungal/bacterial biomass was not significantly different between treatments ($P > 0.05$; Figure 87) and locations ($P > 0.05$). On average fungal biomass and fungal/bacterial biomass ratio of the traditional method was lower than the cover crop treatments; however, the difference resulted not significantly different.

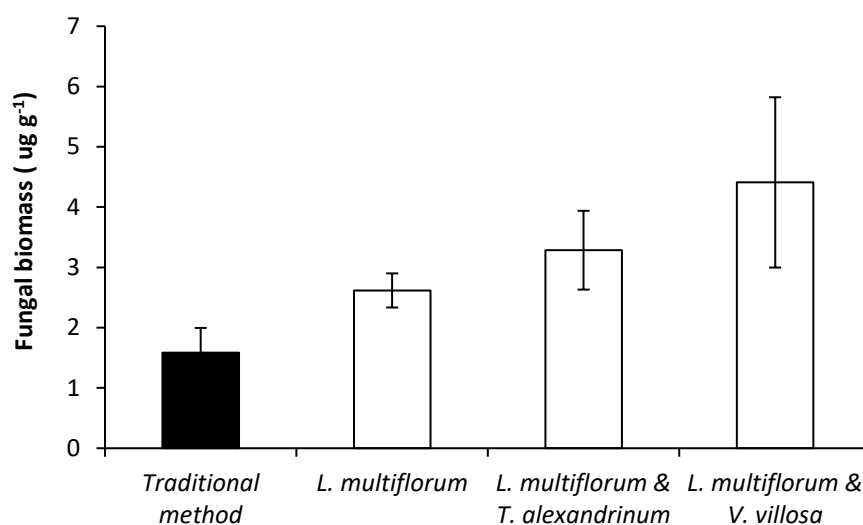


Figure 86 Comparison of fungal biomass between cover crop treatments (white) and the traditional method (black), in March 2016. The traditional method= no cover crops, mouldboard. Vertical bars represent SE. Traditional method, *L. multiflorum* & *T. alexandrinum*, *L. multiflorum* & *V. villosa* n=5; *L. multiflorum* (n=3).

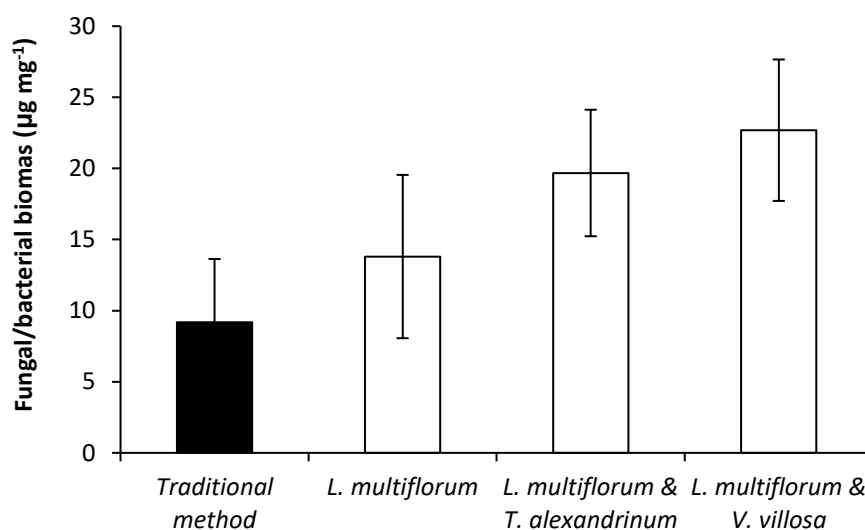


Figure 87 Comparison of fungal/bacterial biomass (µg mg⁻¹) between cover crop treatments (white) and the traditional method (black) in March 2016. The traditional method= no cover crops, mouldboard. Vertical bars represent SE. Traditional method and *L. multiflorum* & *T. alexandrinum*, n=5; *L. multiflorum* & *V. villosa* n=4; *L. multiflorum* n=3.

3.3.3.4 Glomalin related soil proteins

The average recovery for glomalin analysis was 62%. No significant difference was found between treatments ($P>0.05$; Figure 88) and locations ($P>0.05$). On average, glomalin levels of the traditional method were larger than that of the cover crop treatments; yet, the difference resulted not significantly different.

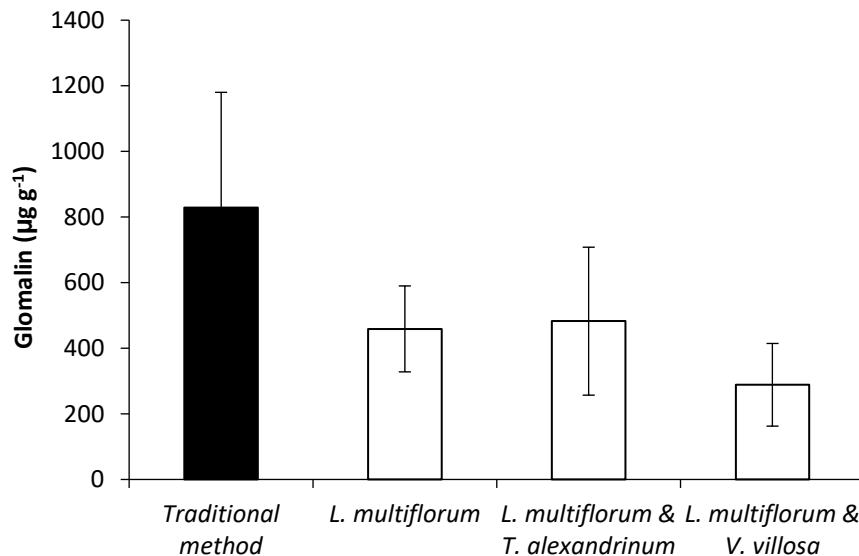


Figure 88 Comparison of glomalin between cover crop treatments (white) and the traditional method (black) in March 2016. The traditional method= no cover crops, mouldboard. Vertical bars represent SE. Traditional method, *L. multiflorum* & *T. alexandrinum* and *L. multiflorum* & *V. villosa* n=4; *L. multiflorum* n=2.

3.3.3.5 Metabolic and microbial quotients

Metabolic and microbial quotients were not significantly affected by treatments ($P>0.05$; Figures 89 and 90), sampling date and location ($P>0.05$). Additionally, there was not distinct difference in metabolic and microbial quotients between the traditional method and the cover crop treatments (Figures 89 and 90).

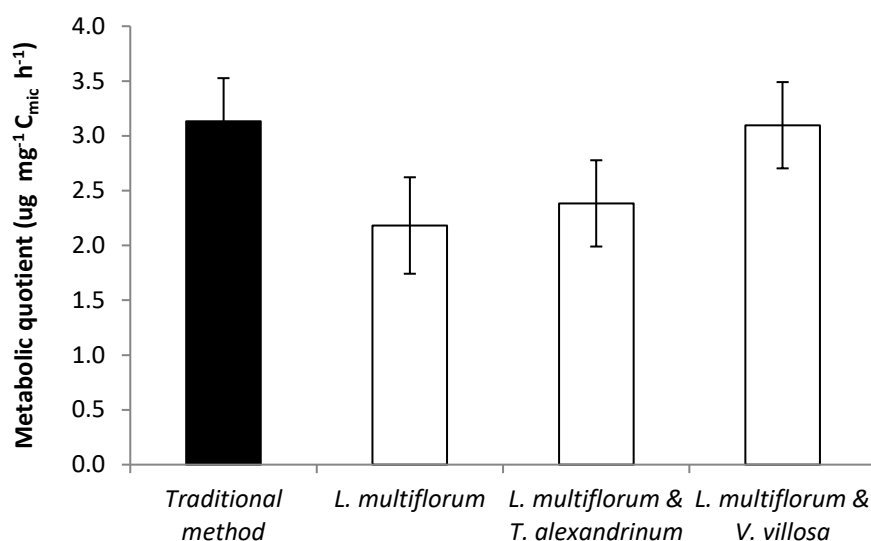


Figure 89 Comparison of metabolic quotient between cover crop treatments (white) and the traditional method (black), two sampling points, November 2015 and March 2016. Vertical bars represent SE (n=10). Traditional method= no cover crops, mouldboard plough.

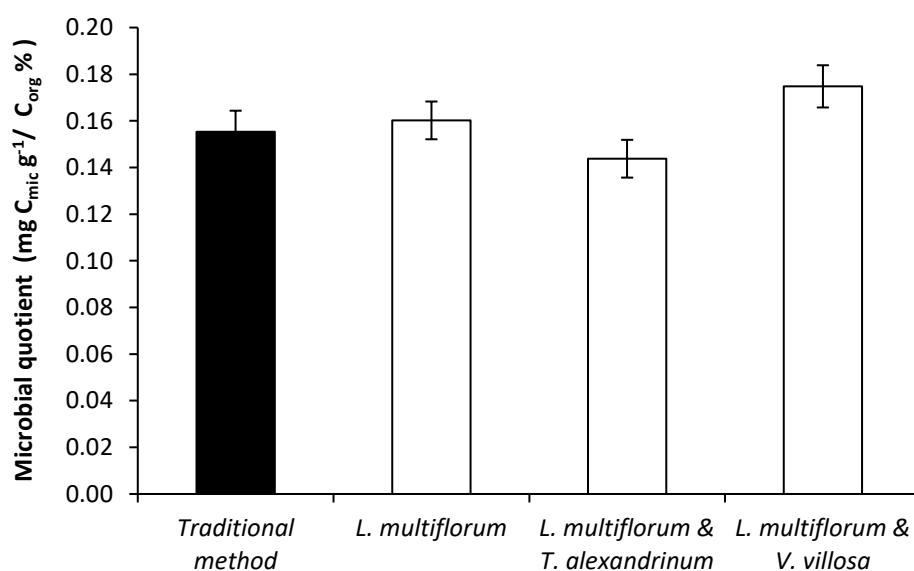


Figure 90 Comparison of microbial quotient (mg C_{mic} g⁻¹ / C_{org} %) between cover crop treatments (white) and the traditional method (black), two sampling points, November 2015 and March 2016. Vertical bars represent SE (n=10). Traditional method= no cover crops, mouldboard plough.

3.3.4 Soil chemistry

Imposed treatments did not affect most chemical parameters, except for EC (Table 19). Total nitrogen, TOC, AP, EC and pH significant diverged between sampling dates (Table 19). The combination between treatment and sampling date did not affect any soil property; while location affected all properties, except for EC (Table 19).

Table 19 Summary of soil chemistry ANOVA P values from Experiment 2.

Soil property	Imposed treatments	Sampling date	P	
			Imposed treatments x Sampling date	Location
OM	>0.05	>0.05	>0.05	<0.01
TN	>0.05	<0.001	>0.05	<0.001
TOC	>0.05	<0.001	>0.05	<0.001
AP	>0.05	<0.05	>0.05	<0.001
EC	<0.01	<0.001	>0.05	>0.05
pH	>0.05	<0.001	>0.05	<0.001

AP=Available Phosphorous; TN=Total Nitrogen; TC=Total Carbon; TOC= Total organic Carbon; EC=Electrical conductivity; OM=Organic matter.

3.3.4.1 Organic matter

Imposed treatments and sampling date did not affect OM ($P>0.05$; Figure 91). Additionally, there was not distinct difference in OM between the traditional method and the cover crop treatments (Figure 91). However, OM in location A was 22% larger compared to the one in B ($P<0.01$; Figure 92). Conversely, there were no differences between B and C, and between A and C.

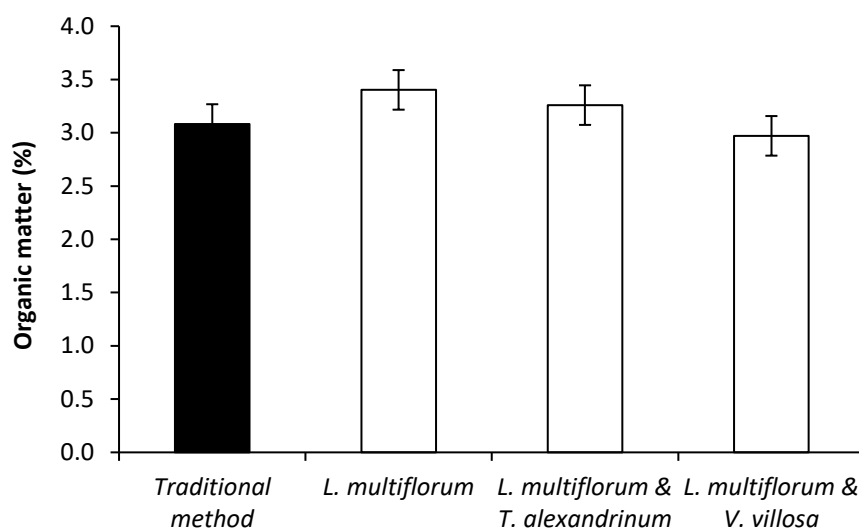


Figure 91 Comparison of organic matter (%) between cover crop treatments (white) and the traditional method (black), two sampling points, November 2015 and March 2016. Vertical bars represent SE (n=10). Traditional method= no cover crops, mouldboard plough.

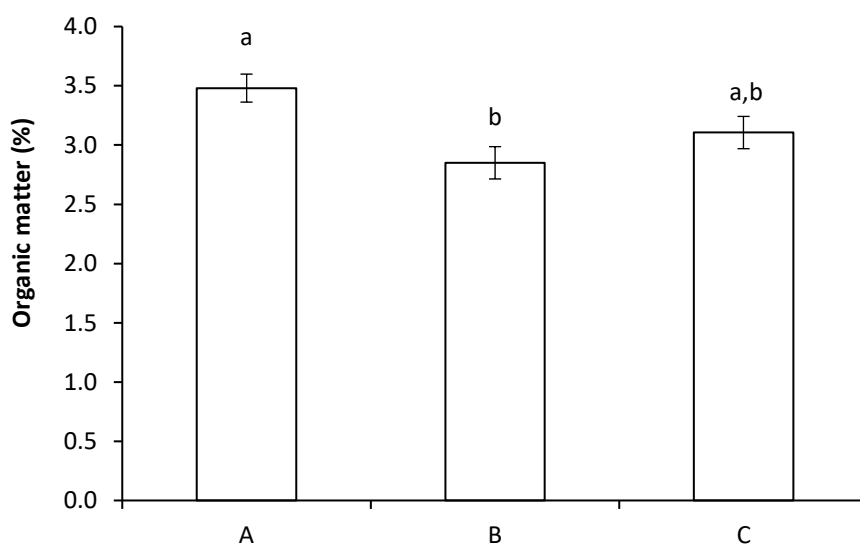


Figure 92 Comparison of organic matter (%) between locations; two sampling points, November 2015 and March 2016. Vertical bars represent SE. A n=16, B n=12, C n=12. Lower case letters represent homogenous groupings. A= west plots; B= central plots (depression); C= east plots. Traditional method= no cover crops, mouldboard plough.

3.3.4.2 Total nitrogen

Quantities of TN were not affected by treatments ($P>0.05$; Figure 93). However, significant differences were found between locations ($P<0.001$). Location A had 22% larger TN compared to B and 14% larger compared to C ($P<0.001$; Figure 94). Yet, there was no difference between B and C ($P<0.05$; Figure 94). In November 2015, TN was 7% larger compared to March 2016 ($P<0.001$; Figure 95). Although, TN was not affected by the interaction between sampling date and imposed treatments ($P>0.05$).

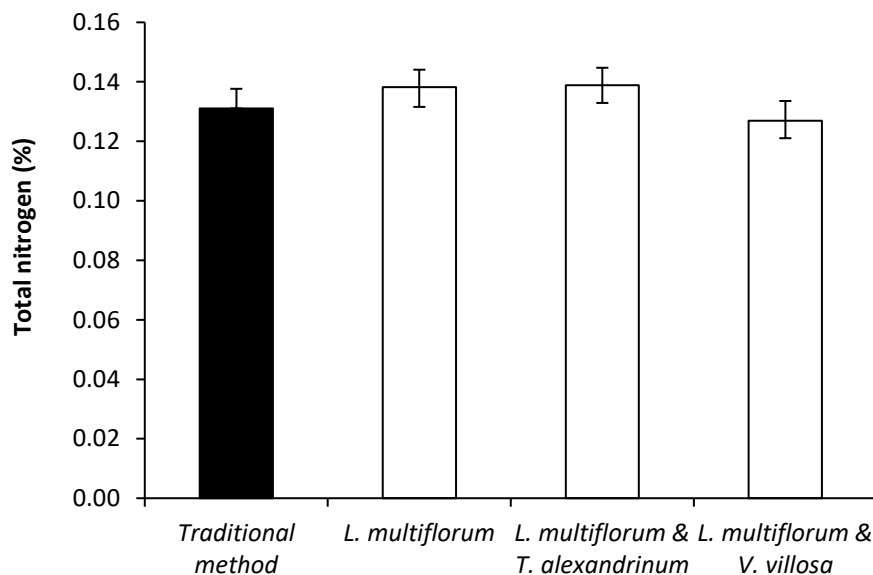


Figure 93 Comparison of total nitrogen (%) between cover crop treatments (white) and the traditional method (black); two sampling points, November 2015 and March 2016. Vertical bars represent SE (n=10). Traditional method= no cover crops, mouldboard plough.

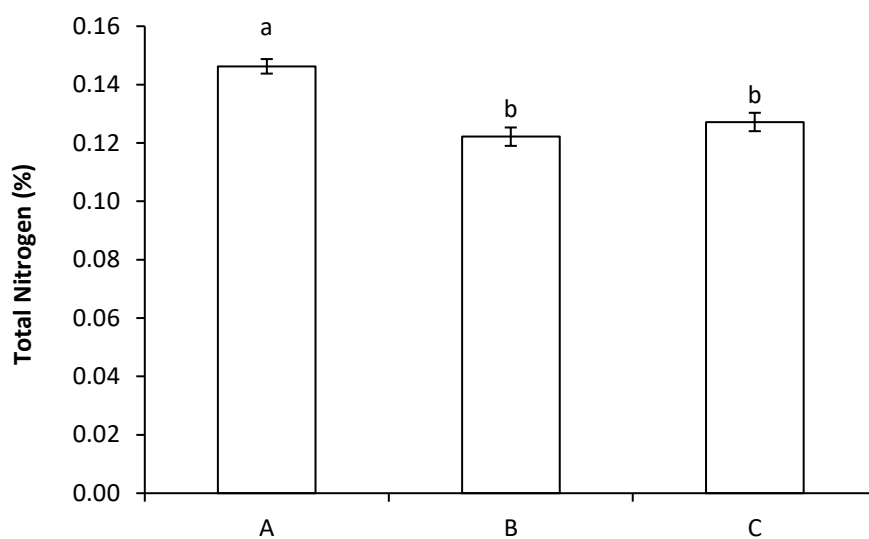


Figure 94 Comparison of total nitrogen (%) between areas; two sampling points, November 2015 and March 2016. Vertical bars represent SE. A n=16, B n=12, C n=12. Lower case letters represent homogenous groupings. A= west plots; B= central plots (depression); C= east plots.

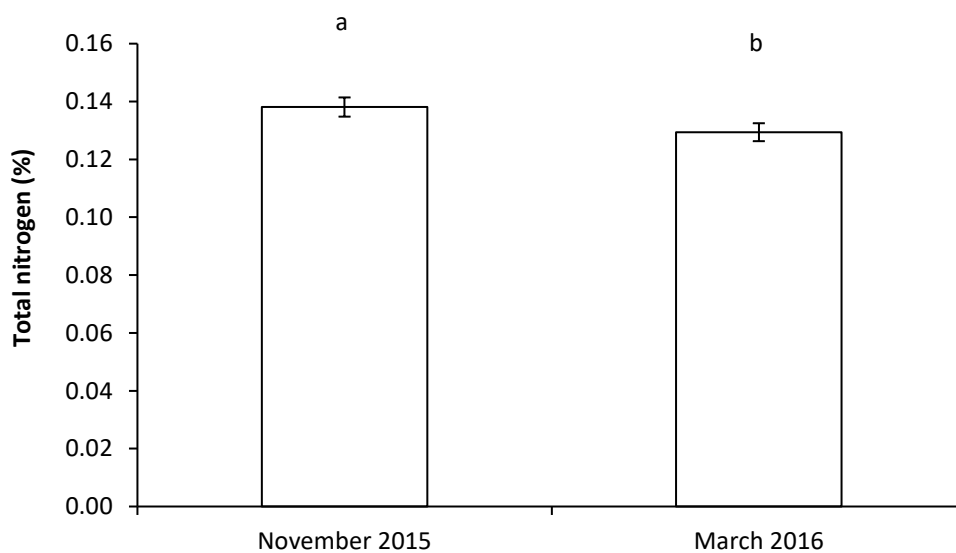


Figure 95 Comparison of total nitrogen in November 2015 and March 2016. Vertical bars represent SE (n=20). Letters represent homogenous groupings.

3.3.4.3 Total organic carbon

Quantities of TOC were not significantly affected by imposed treatments ($P>0.05$; Figure 96) and there was no significant interaction between sampling date and imposed treatments ($P>0.05$). However, TOC in November 2015 was 4% larger than in March 2016 ($P<0.001$; Figure 97). Moreover, samples collected in location A had 15% larger TOC compared to B and 7% larger compared to C (Figure 88); yet, TOC was not significantly affected by the interaction between imposed treatments and location ($P>0.05$).

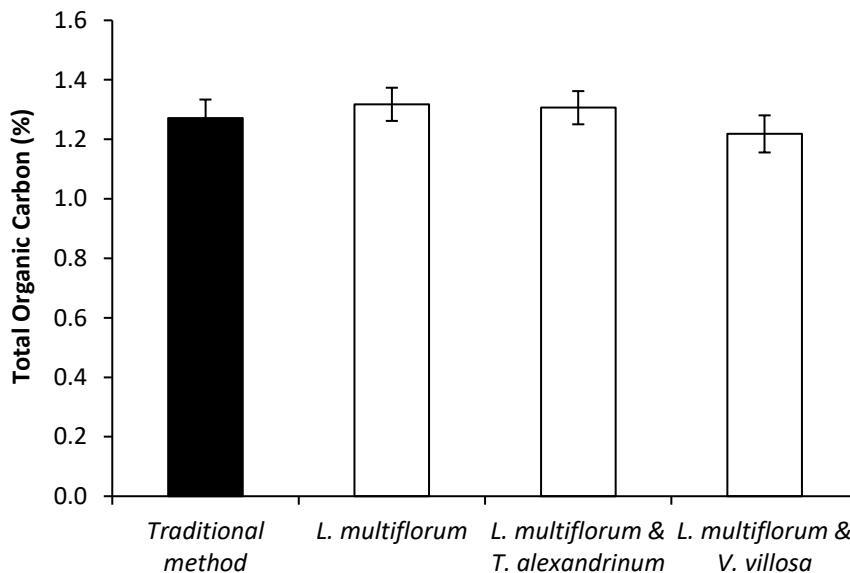


Figure 96 Comparison of total organic carbon (%) between cover crop treatments (white) and the traditional method (black); two sampling points, November 2015 and March 2016. The traditional method= no cover crops, mouldboard. Vertical bars represent SE (n=10).

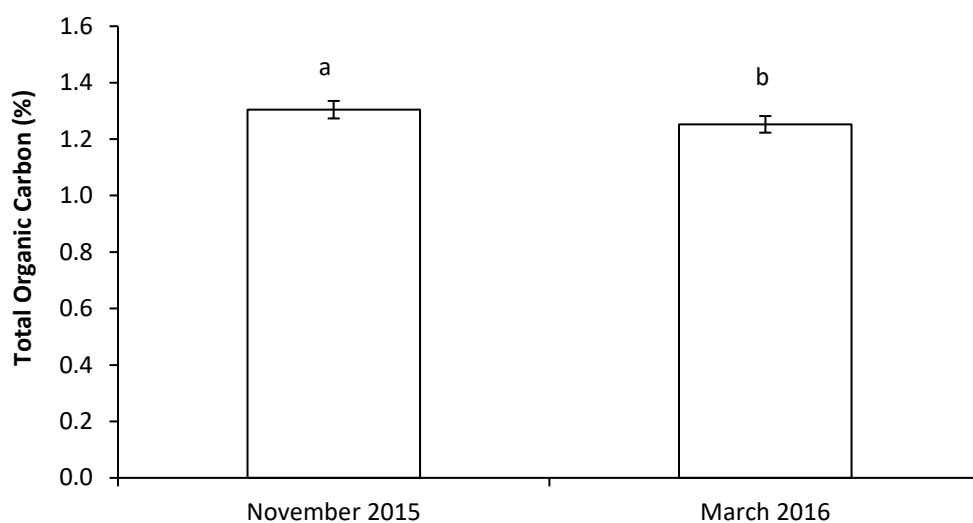


Figure 97 Comparison of total organic carbon in November 2015 and March 2016. Vertical bars represent SE (n=20). Letters represent homogenous groupings.

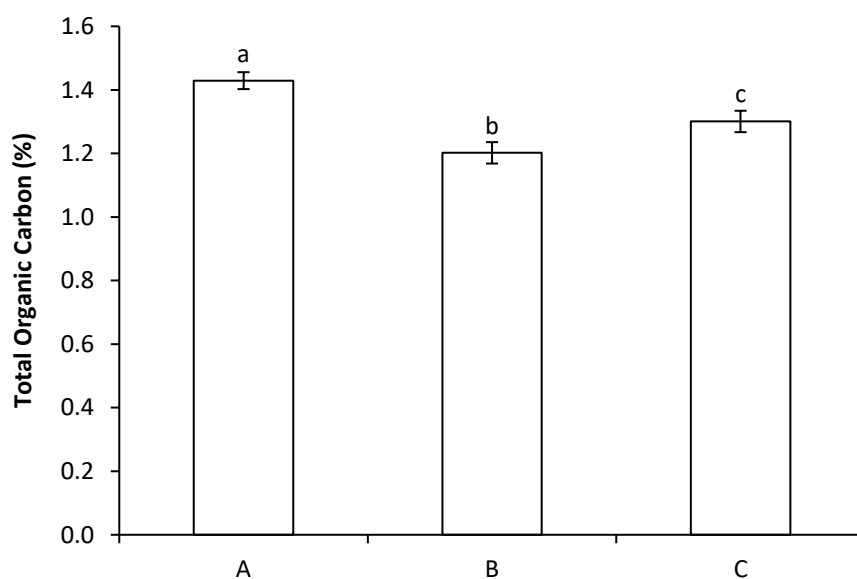


Figure 98 Comparison of total organic carbon between locations; two sampling points, November 2015 and March 2016. Vertical bars represent SE (A n=16, B n=12, C n=12). Lower case letters represent homogenous groupings. A= west plots; B= central plots (depression); C= east plots.

3.3.4.4 Available phosphorous

Cover crop treatments did not affect AP ($P < 0.05$; Figure 99), but AP increased by 11% from November 2015 to March 2016 ($P < 0.05$; Figure 100). However, there was no significant interaction between sampling date and imposed treatments ($P > 0.05$). Sampling location significantly affected AP ($P < 0.001$). Samples collected in location A had 31% less AP compared to B and C ($P < 0.001$; Figure 101). Moreover, the interaction between sampling location and sampling date was significant ($P < 0.05$; Figure 102). In November 2015, in locations B and C, AP was 35% and 20% larger than in A respectively. In March 2016, in locations B and C, AP was 39% and 40% larger than A respectively.

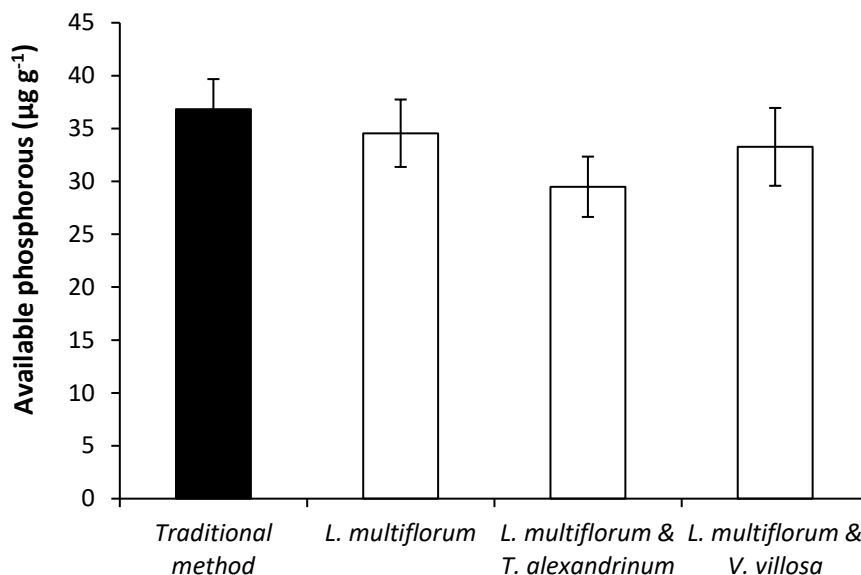


Figure 99 Comparison of available phosphorus ($\mu\text{g g}^{-1}$) between cover crop treatments (white) and the traditional method (black); two sampling points, November 2015 and March 2016. The traditional method= no cover crops, mouldboard. Vertical bars represent SE ($n=10$).

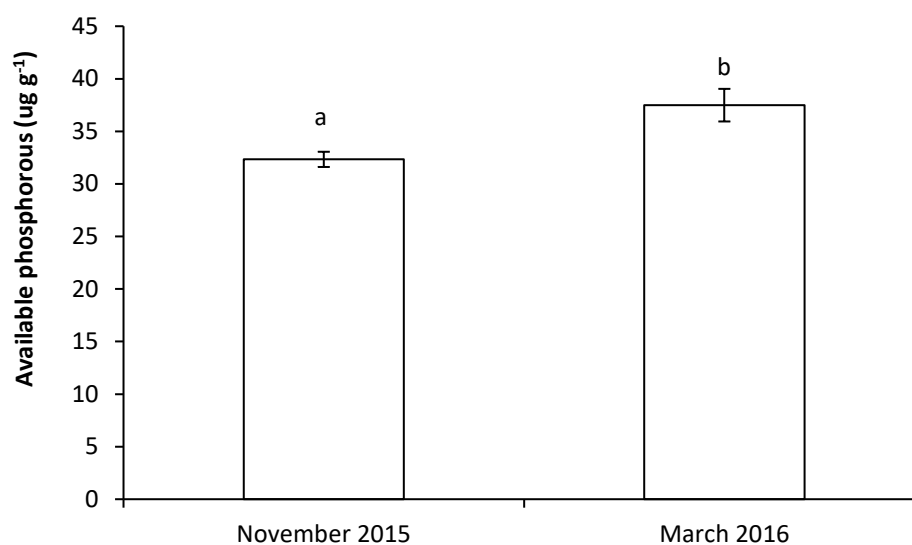


Figure 100 Comparison of available phosphorus ($\mu\text{g g}^{-1}$) measured in November 2015 and March 2016. Vertical bars represent SE ($n=20$). Letters represent homogenous groupings.

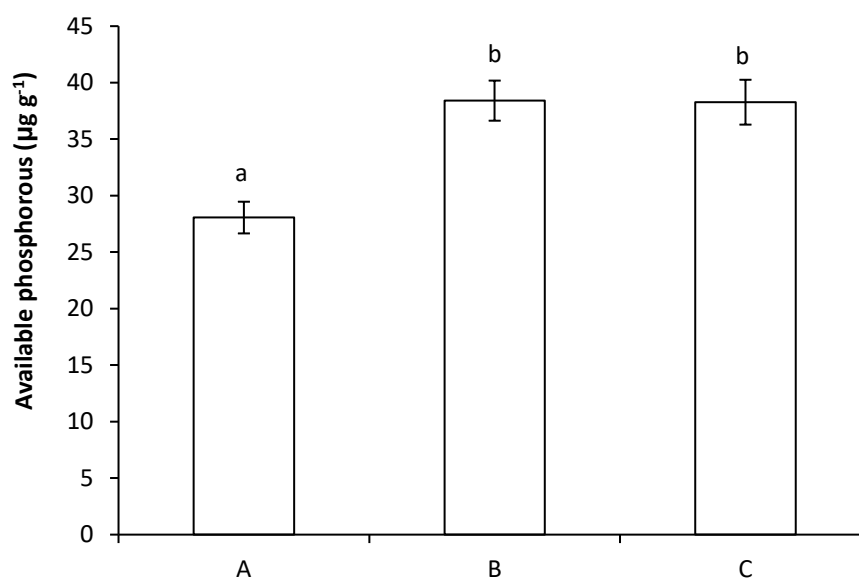


Figure 101 Comparison of available phosphorus ($\mu\text{g g}^{-1}$) between locations; two sampling points, November 2015 and March 2016. Vertical bars represent SE (A $n=16$, B $n=12$, C $n=12$). Lower case letters represent homogenous groupings. A= west plots; B= central plots (depression); C= east plots.

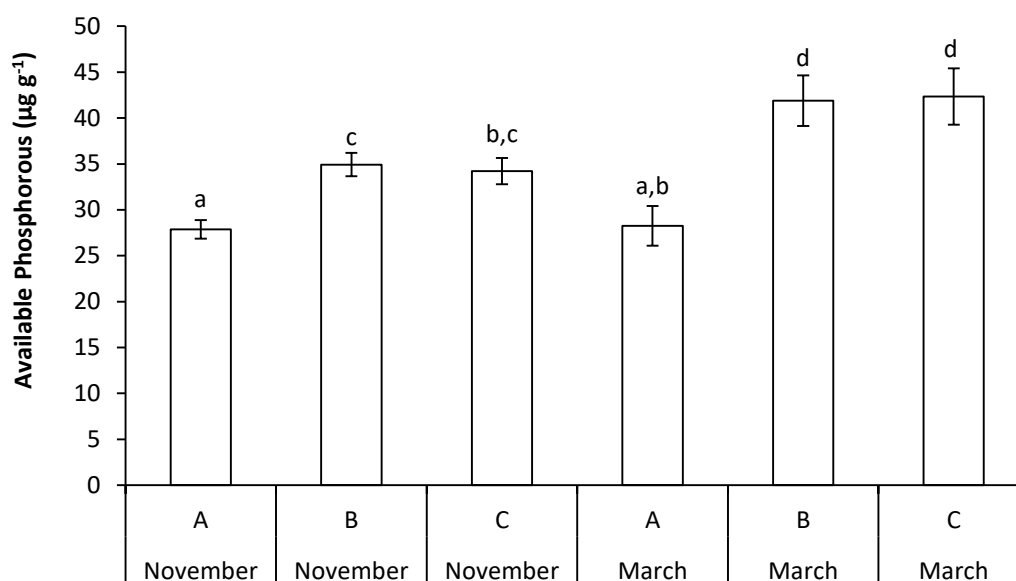


Figure 102 Comparison of available phosphorus between locations measured in November 2015 and in March 2016. Vertical bars represent SE (A n=8, B n=6, C n=6). Lower case letters represent homogenous groupings. A= west plots; B= central plots (depression); C= east plots.

3.3.4.5 Electrical conductivity and pH

Imposed treatments significantly affected EC ($P < 0.01$). Electrical conductivity in the traditional method was 22% lower compared to *L. multiflorum*, 1% lower compared to *L. multiflorum* & *T. alexandrinum*, 17% lower compared to *L. multiflorum* & *V. villosa* ($P < 0.01$; Figure 103). Conversely, there were no differences within the cover crop treatments.

Electrical conductivity decreased by 26% from November 2015 to March 2016 ($P < 0.001$; Figure 104). Average EC_{1-5} measured in Experiment 2 (two sampling points, November 2015 and March 2016) was $49.0 \pm 1.7 \mu S cm^{-1}$, which correspond to an EC_{sa} of $0.52 dS m^{-1}$.

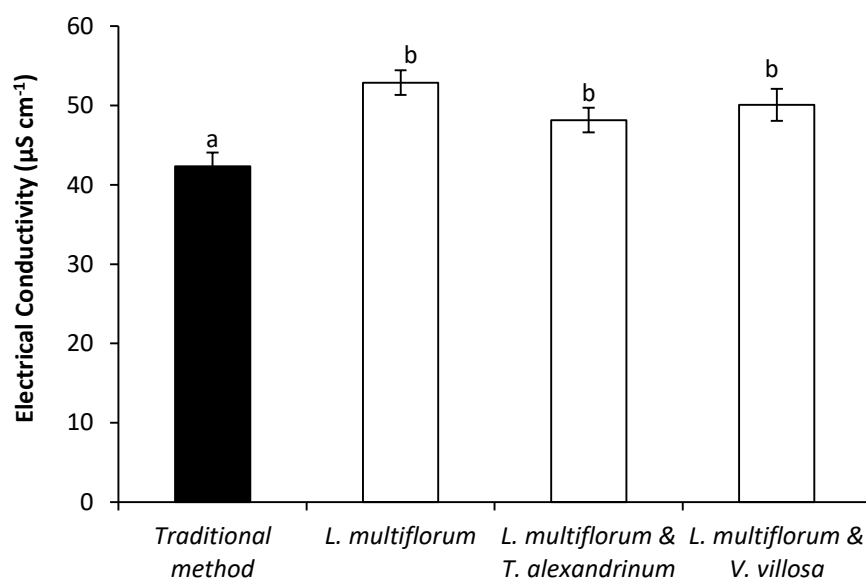


Figure 103 Comparison of electrical conductivity ($\mu\text{S cm}^{-1}$) between cover crops treatments (white) and the traditional method (black); two sampling points, November 2015 and March 2016. The traditional method= no cover crops, mouldboard. Vertical bars represent SE (n=10).

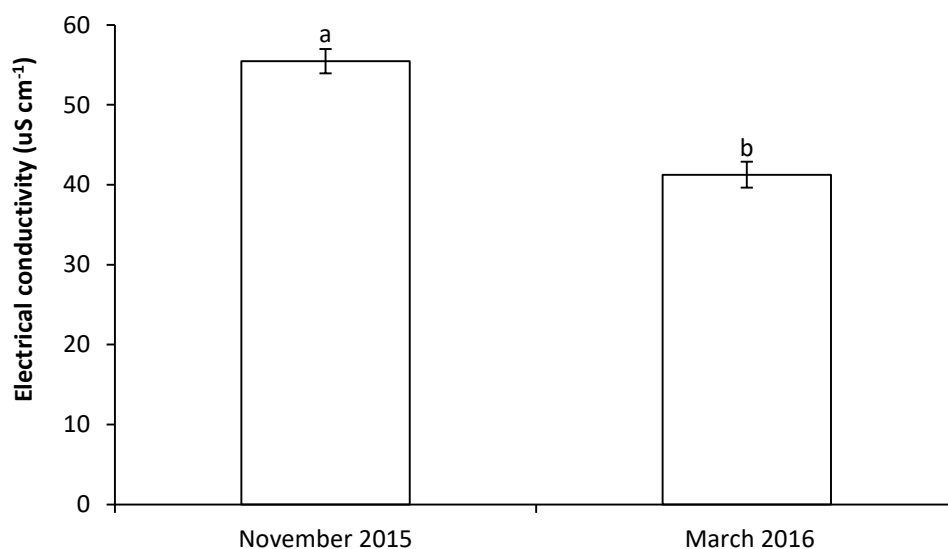


Figure 104 Comparison of electrical conductivity ($\mu\text{S cm}^{-1}$) measured in November 2015 and March 2016. Vertical bars represent SE (n=20), letters represent homogenous groupings.

Soil pH was not significantly affected by treatments ($P>0.05$; Figure 105). Although, it was affected by sampling time ($P<0.001$). In November 2015 soil pH was 5% larger compared to March 2016 ($P<0.001$; Figure 106). Imposed treatments x sampling date did not affect pH ($P>0.05$). Significant differences were found between sampling locations ($P<0.001$; Figure 107). pH measured in location A was 3% lower than in locations B and C. No differences were found between B and C.

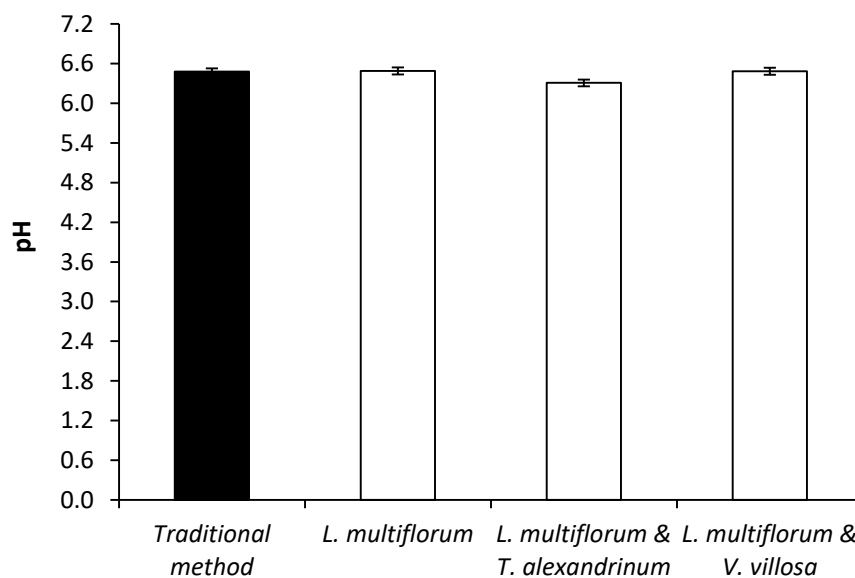


Figure 105 Comparison of pH between cover crops treatments (white) and the traditional method (black); two sampling points, November 2015 and March 2016. The traditional method= no cover crops, mouldboard. Vertical bars represent SE (n=10).

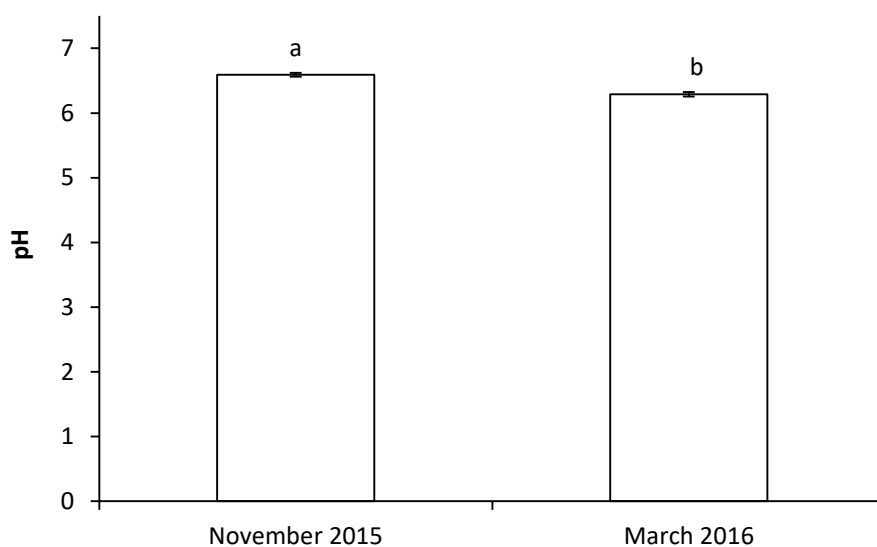


Figure 106 Comparison of pH measured in November 2015 and March 2016. Vertical bars represent SE (n=20), letters represent homogenous groupings.

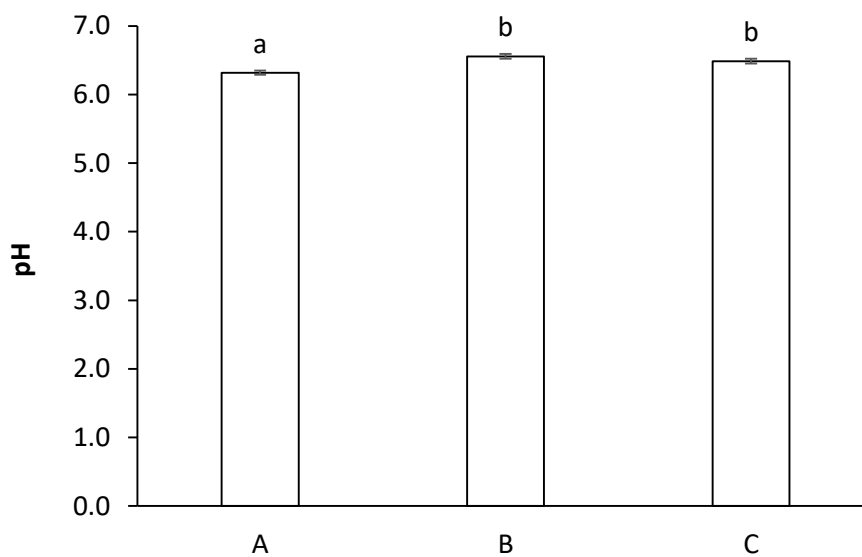


Figure 107 Comparison of soil pH between locations; two sampling points, November 2015 and March 2016. Vertical bars represent SE (A n=16, B n=12, C n=12). Lower case letters represent homogenous groupings. A= west plots; B= central plots (depression); C= east plots.

3.3.5 Soil physics

3.3.5.1 Aggregate stability

The percentage of unstable aggregates was not significantly different between treatments ($P>0.05$) for neither the 1-10 years return storm (Figure 108), nor for the 1-50 years (Figure 109). Additionally, sampling location did not affect any parameter ($P>0.05$).

Regarding the 1-10 years return storm, on average, the traditional method had the lower percentage of unstable aggregates and *L. multiflorum* the greatest (Figure 108). In regards to the cover crop treatments *L. multiflorum* the greatest percentage of unstable aggregates and *L. multiflorum* & *V. villosa* the least. However, the difference was not significant ($P>0.05$). Regarding the 1-50 years return storm, all treatments had on average, 50% unstable aggregates (Figure 109).

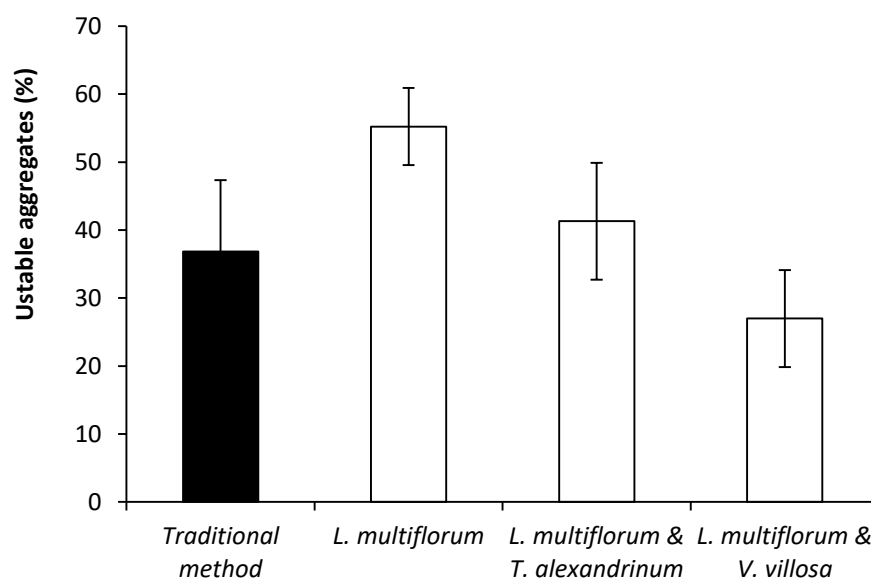


Figure 108 Comparison of unstable aggregates (%) between cover crops treatments (white) and the traditional method (black) for a 1-10 years return storm. Vertical bars represent SE (n=5).

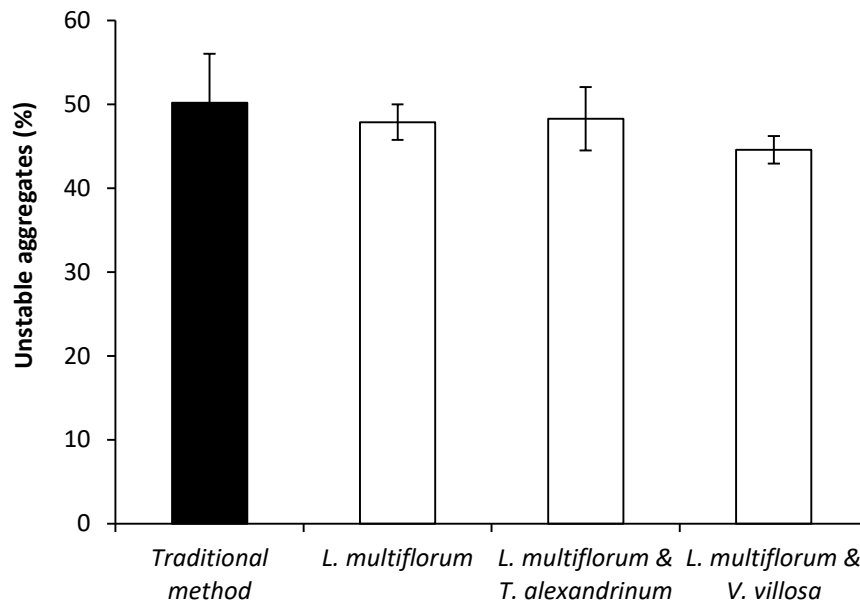


Figure 109 Comparison of unstable aggregates (%) between cover crops treatments (white) and the traditional method (black) for a 1-50 years return storm. Vertical bars represent SE (n=5).

3.3.5.2 Runoff and soil loss

There was no significant difference between any of the treatments for runoff volume for any of the five collection periods ($P > 0.05$; Figures 110-114). Additionally, imposed treatments did not affect overall runoff volume collected during Experiment 2 (cumulative values) ($P > 0.05$; Figures 120).

There was no significant difference in runoff volume between the treatments during the first collection period, 23/07/2015 - 15/10/2015, during which maize was still present in the plots (Figure 110). The average of runoff collected in the treatment *L. multiflorum* & *T. alexandrinum* was larger compared to the rest of the treatments. However, the difference was not significant due to major deviation (SE) within the treatments. In regards of the cover crop treatments *L. multiflorum* & *T. alexandrinum* had on average the largest runoff production and

L. multiflorum and *L. multiflorum* & *V. villosa* had similar average levels of runoff.

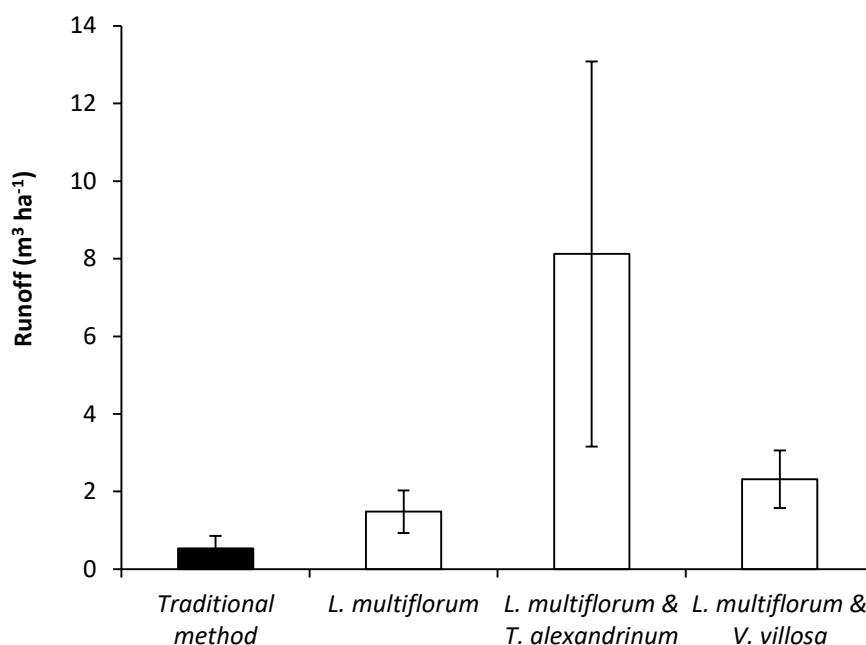


Figure 110 Comparison of runoff ($\text{m}^3 \text{ ha}^{-1}$) between cover crop treatments (white) and the traditional method (black) collected between 23/07/2015 and 15/10/2015. Vertical bars represent SE ($n=5$). Traditional method= no cover crops, mouldboard plough.

There was no significant difference in runoff volume between the treatments during the second collection period (27/11/2015 - 21/12/2015; Figure 111). The traditional method (no cover crop) had on average greater runoff production compared to the cover crop treatments. However, the difference was not significant due to major variation (SE) observed within the treatments. In regards to the cover crop treatments, no differences were found and on average the cover crop had similar levels of runoff volumes.

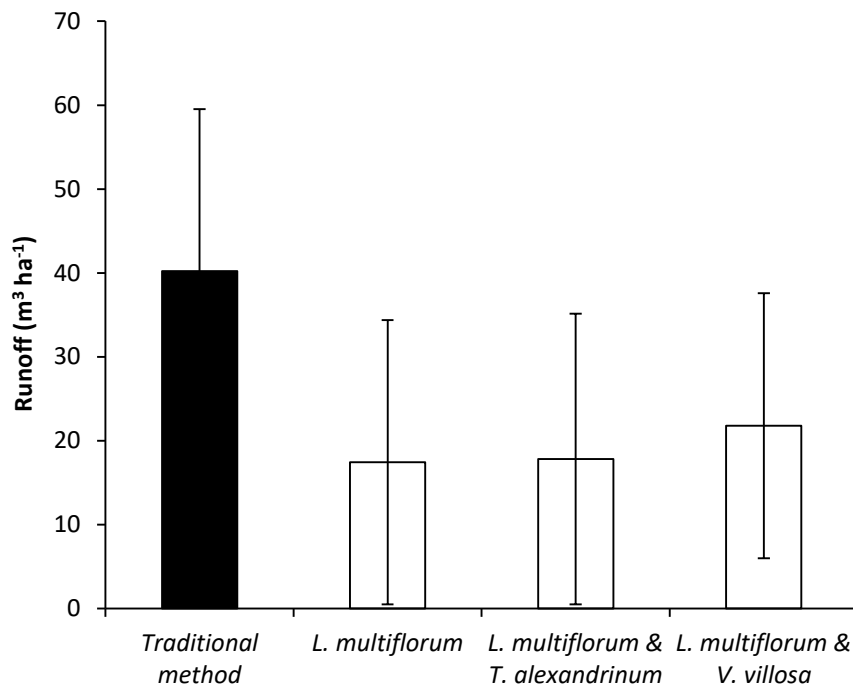


Figure 111 Comparison of runoff ($\text{m}^3 \text{ha}^{-1}$) between cover crop treatments (white) and the traditional method (black) collected between 27/11/2015 and 21/12/2015. Vertical bars represent SE ($n=5$). Traditional method= no cover crops, mouldboard plough.

There was no significant difference in runoff volume between the treatments collected in the third collection period (21/12/2015 - 13/01/2016; Figure 112). The average runoff collected in the treatment *L. multiflorum* & *V. villosa* was lower compared to the rest of the treatments. The traditional method and *L. multiflorum* & *T. alexandrinum* had the greatest average runoff production. However, the differences were not significant due to major variation (SE) within the treatments. In regards to the cover crop treatments, *L. multiflorum* & *T. alexandrinum* had on average the greatest runoff production, while *L. multiflorum* & *V. villosa* and *L. multiflorum* had similar average levels.

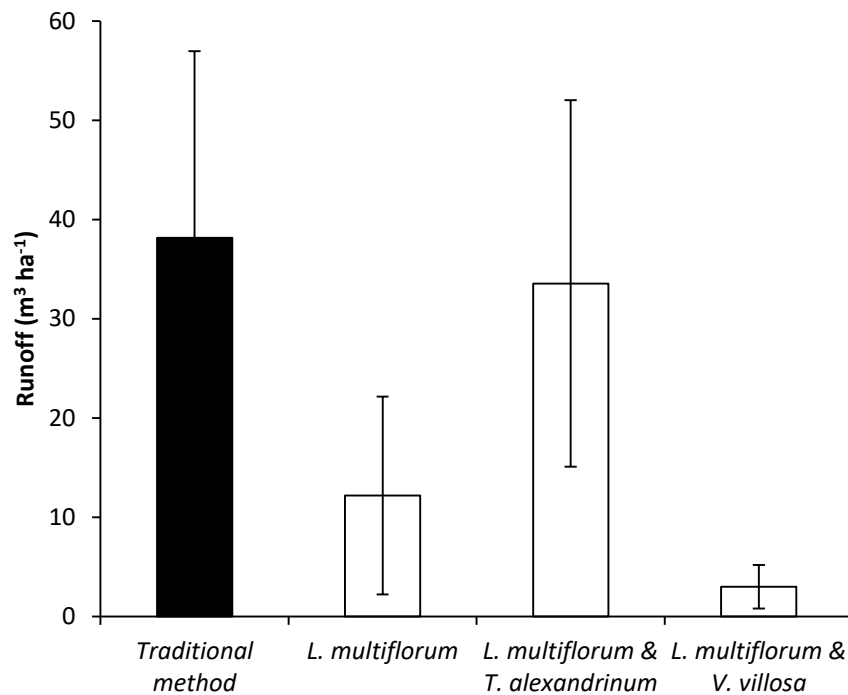


Figure 112 Comparison of runoff ($\text{m}^3 \text{ha}^{-1}$) between cover crop treatments (white) and the traditional method (black) collected between 21/12/2015 and 13/01/2016. Vertical bars represent SE ($n=5$). Traditional method= no cover crops, mouldboard plough.

There was no significant difference in runoff volume between the treatments during the fourth collection period, (13/01/2016 and 16/02/2016; $P>0.05$; Figure 113). Major variation (SE) was observed within the treatments, especially in the treatment *L. multiflorum* & *V. villosa*. The traditional method and *L. multiflorum* had similar average level of runoff. In regards to the cover crop treatments, *L. multiflorum* had on average the greatest runoff production, while *L. multiflorum* & *V. villosa* had the lowest average level.

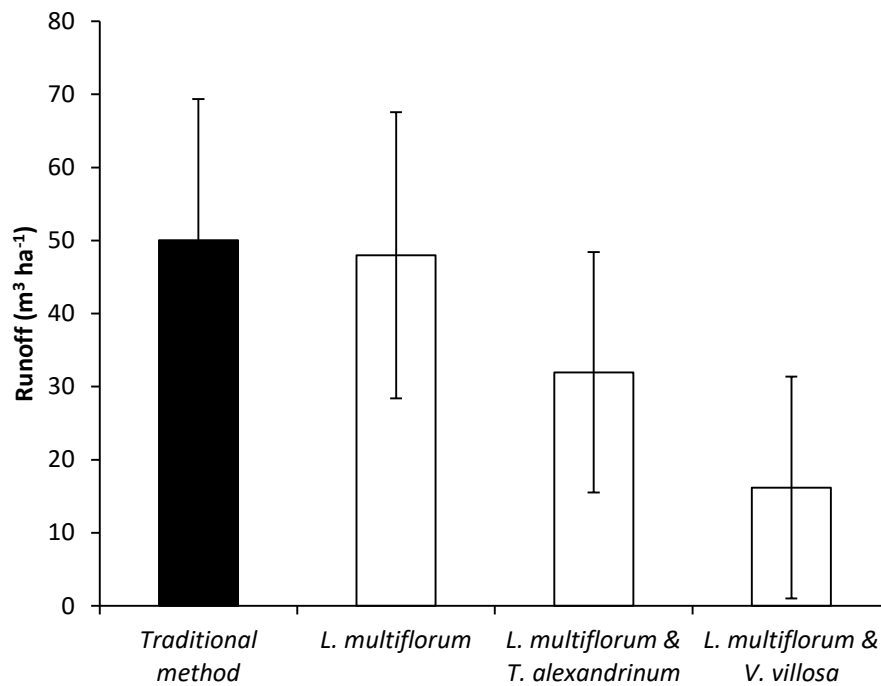


Figure 113 Comparison of runoff ($\text{m}^3 \text{ha}^{-1}$) between cover crop treatments (white) and the traditional method (black) collected between 13/01/2016 and 16/02/2016. Vertical bars represent SE ($n=5$). Traditional method= no cover crops, mouldboard plough.

Runoff volume collected between 16/02/2016 - 15/03/2016 (fifth collection period) was not significantly different between the treatments. ($P>0.05$; Figure 114). Similarly to the previous collection period, major variation (SE) was observed within the treatments, especially in the treatment *L. multiflorum* & *V. villosa*. The traditional method and *L. multiflorum* had similar average level of runoff. In regards to the cover crop treatments, *L. multiflorum* had on average the greatest runoff production, while *L. multiflorum* & *V. villosa* had the lowest average level.

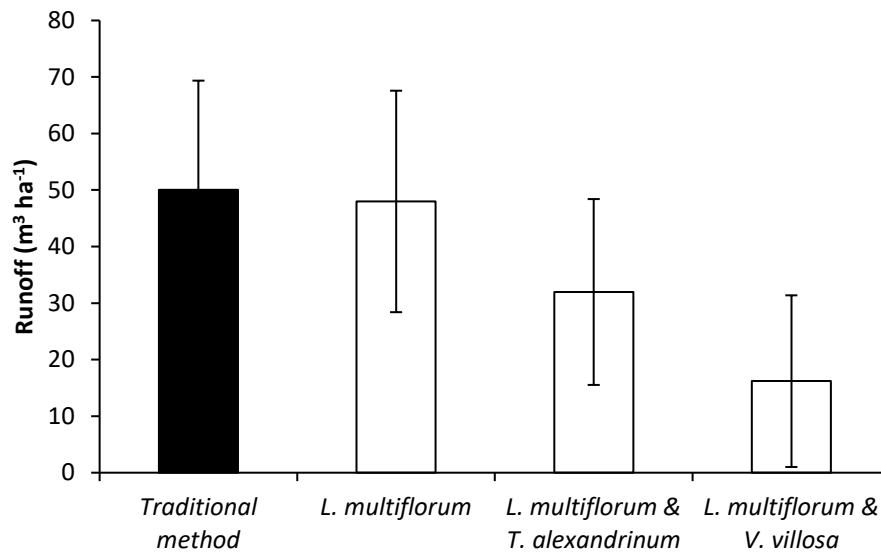


Figure 114 Comparison of runoff ($\text{m}^3 \text{ha}^{-1}$) between cover crop treatments (white) and the traditional method (black) collected between 16/02/2016 and 15/03/2016. Vertical bars represent SE ($n=5$). Traditional method= no cover crops, mouldboard plough.

There was no significant difference between any of the treatments for soil loss for any of the five collection periods ($P > 0.05$; Figures 115-119). Additionally, imposed treatments did not affect overall soil loss collected during Experiment 2 (cumulative values) ($P > 0.05$; Figures 121).

There was no significant difference in soil loss collected in the treatments during the first collection period (23/07/2015 - 15/10/2015) during which maize was still present in the plots (Figure 115). The average soil loss collected in the treatment *L. multiflorum* & *T. alexandrinum* was largest and in the traditional method was the lowest compared to the rest of the treatments. However, the differences were not significant due to major deviation (SE) within the treatments. *L. multiflorum* and the traditional method had similar average levels of soil loss. In regards to the cover crop treatments, *L. multiflorum* & *T. alexandrinum* had the largest soil loss, while *L. multiflorum* and *L. multiflorum* & *V. villosa* had similar levels of soil loss.

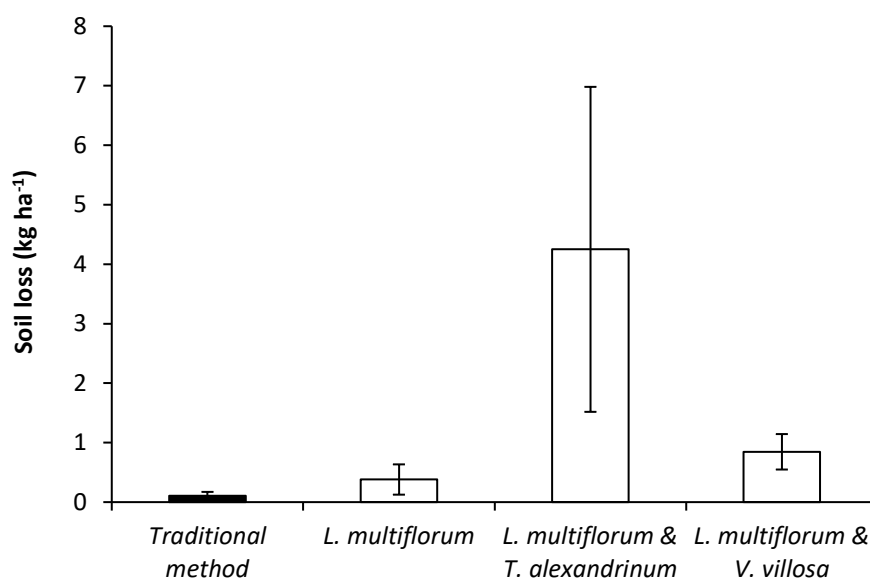


Figure 115 Comparison of soil loss (kg ha⁻¹) between cover crop treatments (white) and the traditional method (black) collected between 23/07/2015 and 15/10/2015. Vertical bars represent SE (n=5). Traditional method= no cover crops, mouldboard plough.

There was no significant difference in soil loss between the treatments during the collection second collection period (27/11/2015 - 21/12/2015; $P>0.05$; Figure 116). Comparable average quantities were collected in each treatment and major variation (SE) was observed within the treatments. No differences were found within the cover crop treatments ($P>0.05$).

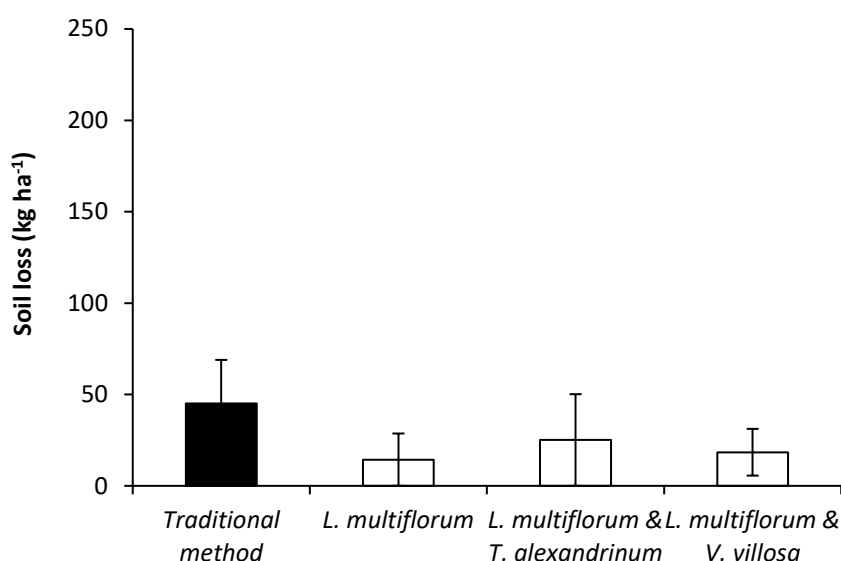


Figure 116 Comparison of soil loss (kg ha⁻¹) between cover crop treatments (white) and the traditional method (black) collected between 27/11/2015 and 21/12/2015. Vertical bars represent SE (n=5). Traditional method= no cover crops, mouldboard plough.

There was no significant difference in soil loss between the treatments during the third collection period (21/12/2015 - 13/01/2016; $P > 0.05$; Figure 117). The average of soil loss in the treatments *L. multiflorum* and *L. multiflorum* & *V. villosa* was lower compared to the traditional method and *L. multiflorum* & *T. alexandrinum*. However, the difference was not significant due to major deviation (SE) within the treatments. No significant differences were found within the cover crop treatments ($P > 0.05$).

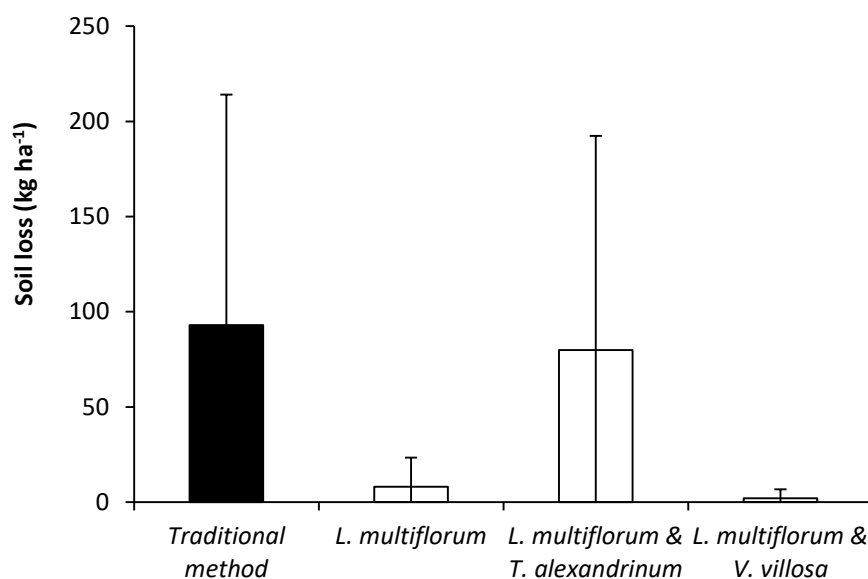


Figure 117 Comparison of soil loss (kg ha⁻¹) between cover crop treatments (white) and the traditional method (black) collected between 21/12/2015 and 13/01/2016. Vertical bars represent SE (n=5). Traditional method= no cover crops, mouldboard plough.

During the collection fourth period (13/01/2016 - 16/02/2016) soil loss was not significantly affected by the treatments ($P>0.05$; Figure 118). Similar average quantities were collected in each treatment and major variation (SE) was observed within the treatments, especially in the treatment *L. multiflorum* & *V. villosa*.

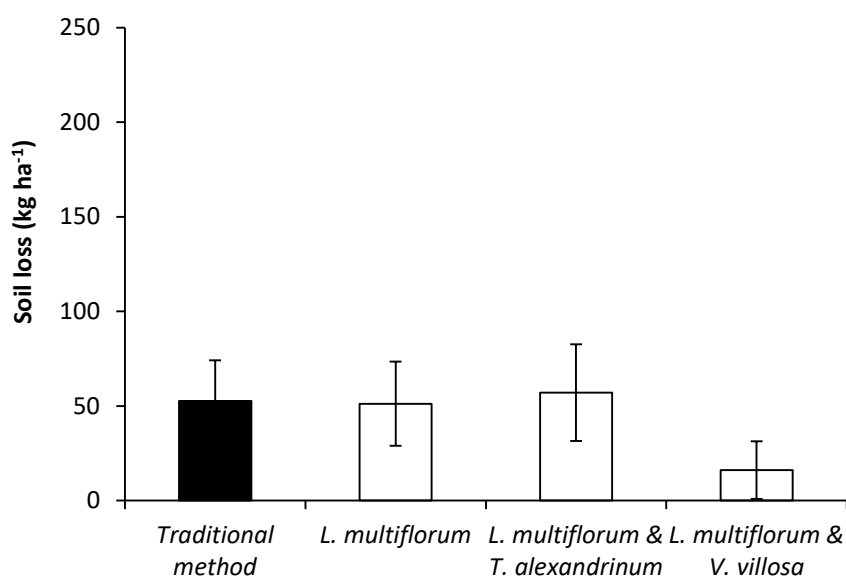


Figure 118 Comparison of soil loss (kg ha⁻¹) between cover crop treatments (white) and the traditional method (black) collected between 13/01/2016 and 16/02/2016. Vertical bars represent SE (n=5). Traditional method= no cover crops, mouldboard plough.

There was no significant difference in soil loss between the treatments during the fifth collection period (16/02/2016 - 15/03/2016; $P > 0.05$; Figure 119). Average soil loss in the treatment *L. multiflorum* & *V. villosa* was lower compared to the rest of the treatments. Although, the difference was not significant due to major deviation (SE) within the treatments. The traditional method, *L. multiflorum* and *L. multiflorum* & *T. alexandrinum* had similar average levels.

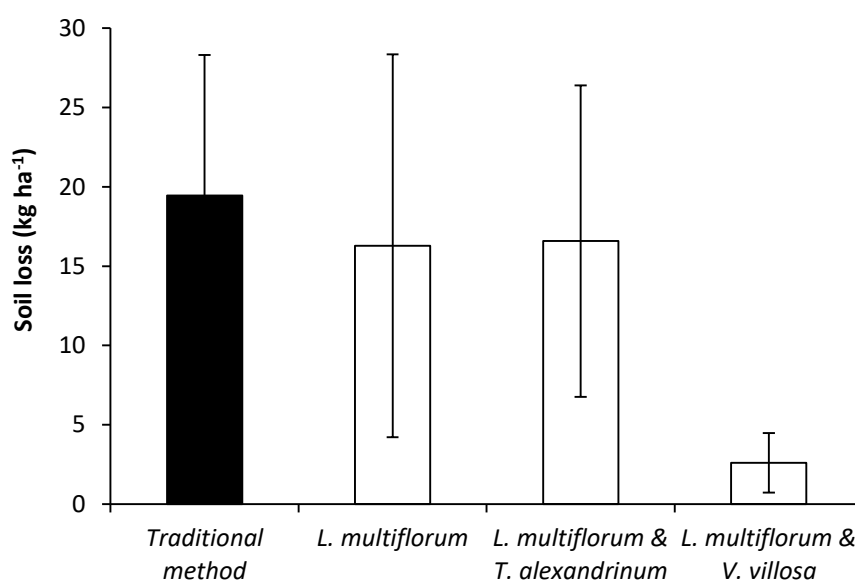


Figure 119 Comparison of soil loss (kg ha⁻¹) between cover crop treatments (white) and the traditional method (black) collected between 16/02/2016 to the 15/03/2016. Vertical bars represent SE (n=5). Traditional method= no cover crops, mouldboard plough.

Over the entire collection period, there were no significant differences in runoff (Figure 120). Average runoff in the traditional method was larger compared to the rest of the treatments. Yet, the difference was not significant due to major deviation (SE) within the treatments. In regards to the cover crop treatments, on average, *L. multiflorum* & *V. villosa* had the lowest runoff production.

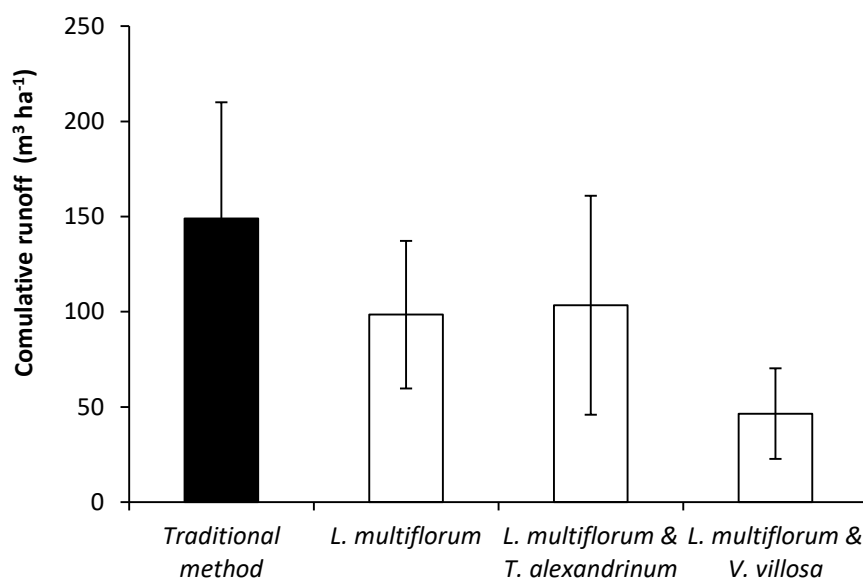


Figure 120 Comparison of cumulative runoff ($\text{m}^3 \text{ha}^{-1}$) (July 2015 - March 2016) between cover crop treatments (white) and the traditional method (black). Vertical bars represent SE ($n=5$). Traditional method= no cover crops, mouldboard plough.

Cumulative soil loss was not significantly different between treatments ($P>0.05$; Figure 121). Average soil loss in the traditional method and in the treatment *L. multiflorum* & *T. alexandrinum* was larger compared to the rest of the treatments. However, the difference was not significant due to major deviation (SE) within the treatments. In regards to the cover crop treatments, on average, *L. multiflorum* & *T. alexandrinum* had the largest runoff production.

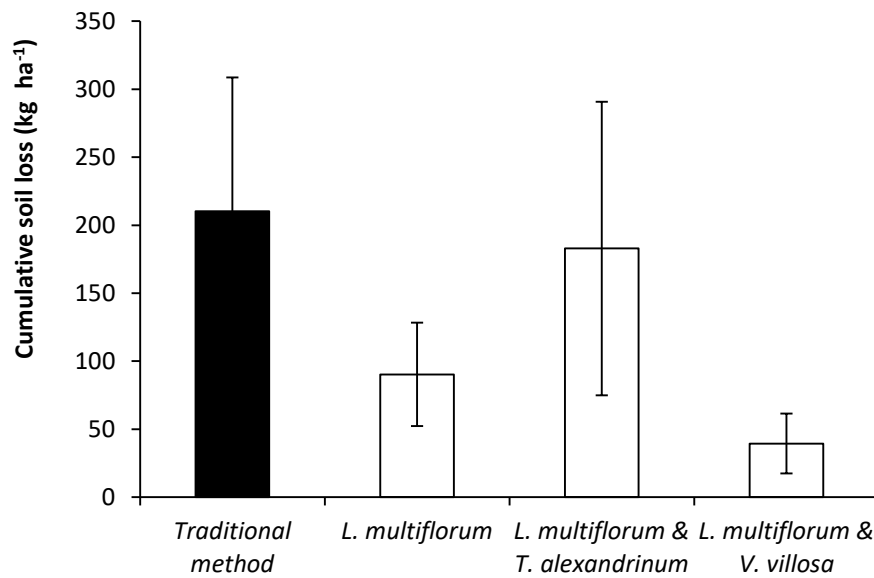


Figure 121 Comparison cumulative of soil loss (kg ha⁻¹) (July 2015 - March 2016) between cover crop treatments (white) and the traditional method (black) Vertical bars represent SE (n=5). Traditional method= no cover crops, mouldboard plough.

3.3.5.2.1 Effect of plot location

Erosion plot location significantly affected both runoff volumes and soil loss quantities in two collection periods and overall in Experiment 2 (cumulative values). During the second collection period (27/11/2015 - 21/12/2015) plots located in B had 82% larger runoff compared to A and 99% larger compared to C ($P < 0.001$; Figure 122). Similarly, during the fourth collection period (13/01/2016 – 16/02/2016), plots located in B had 85% larger runoff compared to C ($P < 0.05$; Figure 123). Conversely, there were no differences between A and C, and between A and B. Cumulative runoff collected in zone B was 56% larger compared to A and 82% larger compared to C ($P < 0.05$; Figure 124). No differences between A and C were recorded.

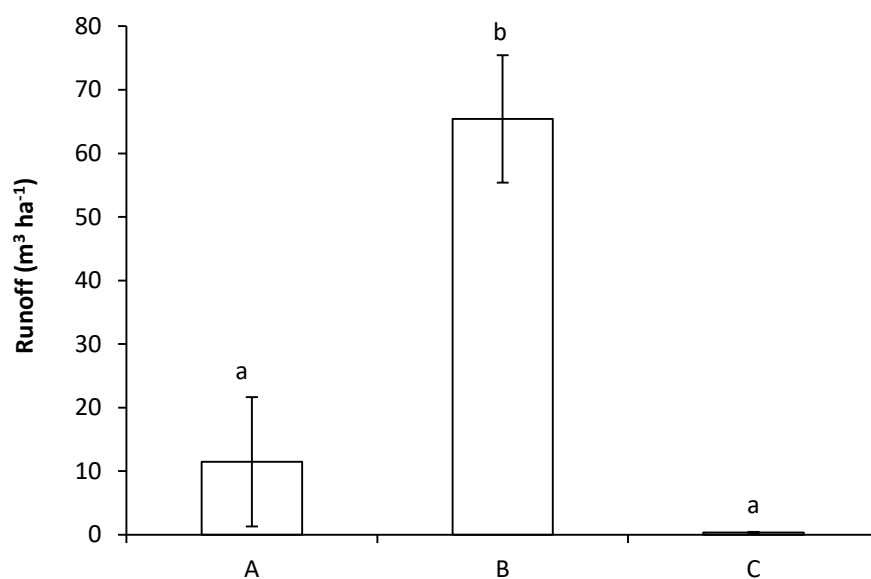


Figure 122 Comparison of runoff ($\text{m}^3 \text{ ha}^{-1}$) between locations (27/11/2015 - 21/12/2015). Vertical bars represent SE. Vertical bars represent SE. A n=8, B n=6, C n=6. Lower case letters represent homogenous groupings. A= west plots; B= central plots (depression); C= east plots.

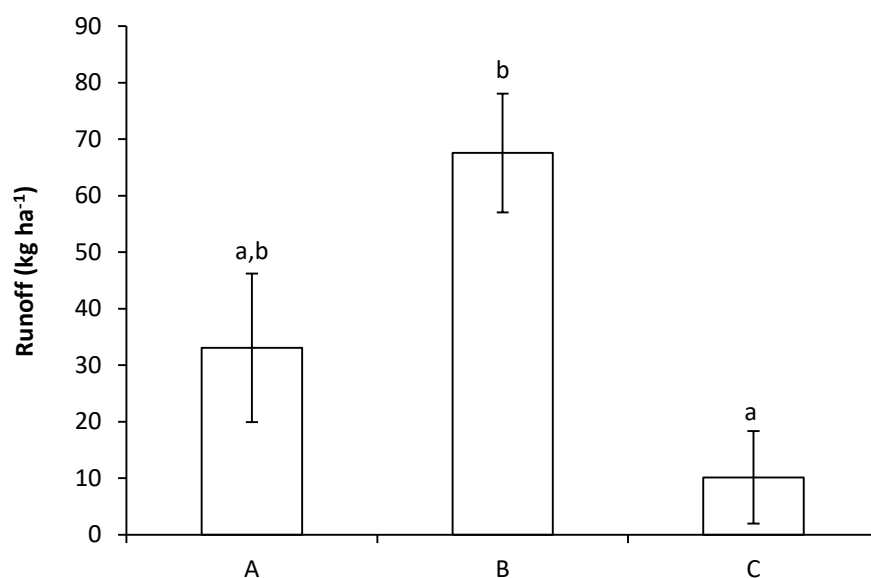


Figure 123 Comparison of runoff ($\text{m}^3 \text{ ha}^{-1}$) between locations (13/01/2016 - 16/02/2016). Vertical bars represent SE. Vertical bars represent SE. A n=8, B n=6, C n=6. Lower case letters represent homogenous groupings. A= west plots; B= central plots (depression); C= east plots.

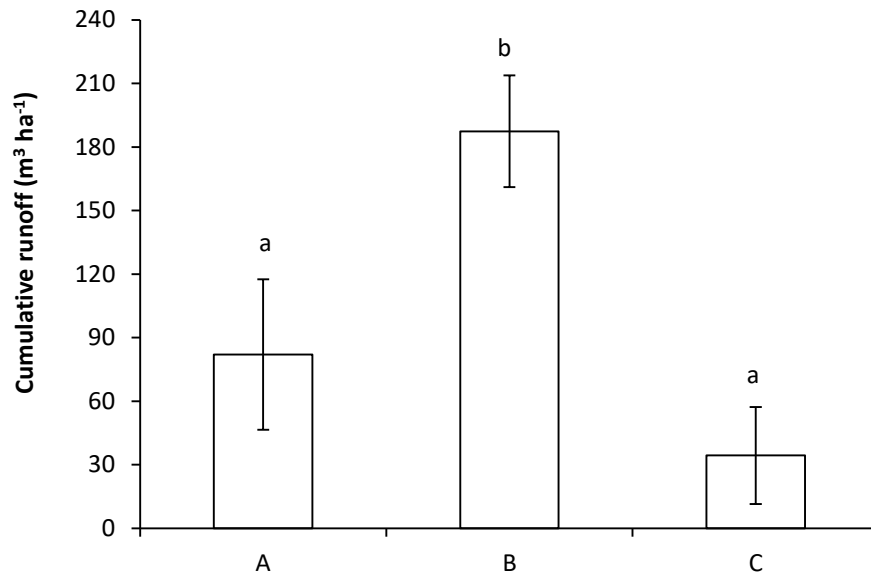


Figure 124 Comparison of cumulative runoff ($\text{m}^3 \text{ ha}^{-1}$) between locations (13/01/2016 - 16/02/2016). Vertical bars represent SE. Vertical bars represent SE. A n=8, B n=6, C n=6. Lower case letters represent homogenous groupings. A= west plots; B= central plots (depression); C= east plots.

Significant differences in soil loss recorded during the second period (27/11/2015 - 21/12/2015) were found between locations ($P < 0.001$). Location C did not produce soil loss. Soil loss collected during in B was 76% larger compared to A and 100% larger compared to C ($P < 0.001$; Figure 125). Although, there were no differences between A and C, due to major deviation (SE) between locations, especially location A. Location also affected soil loss for the entire collection period (cumulative values) ($P < 0.05$; Figure 126). Location B had 67% larger soil loss compared to A and 132% larger compared to C. However, there were no differences between A and C, and between A and B, due to major deviation (SE) between locations, especially location A.

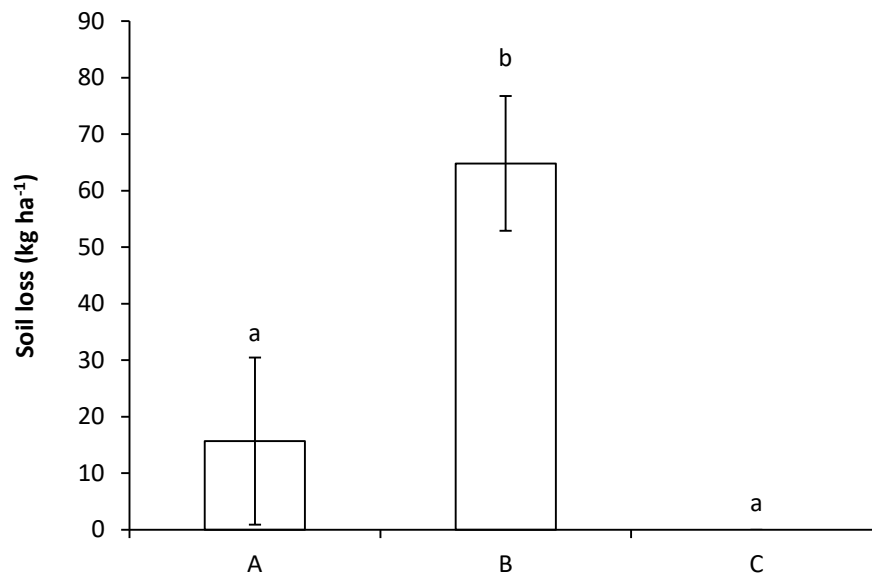


Figure 125 Comparison of soil loss (kg ha⁻¹) between locations (27/11/2015 - 21/12/2015). Vertical bars represent SE. Vertical bars represent SE. A n=8, B n=6, C n=6. Lower case letters represent homogenous groupings. A= west plots; B= central plots (depression); C= east plots.

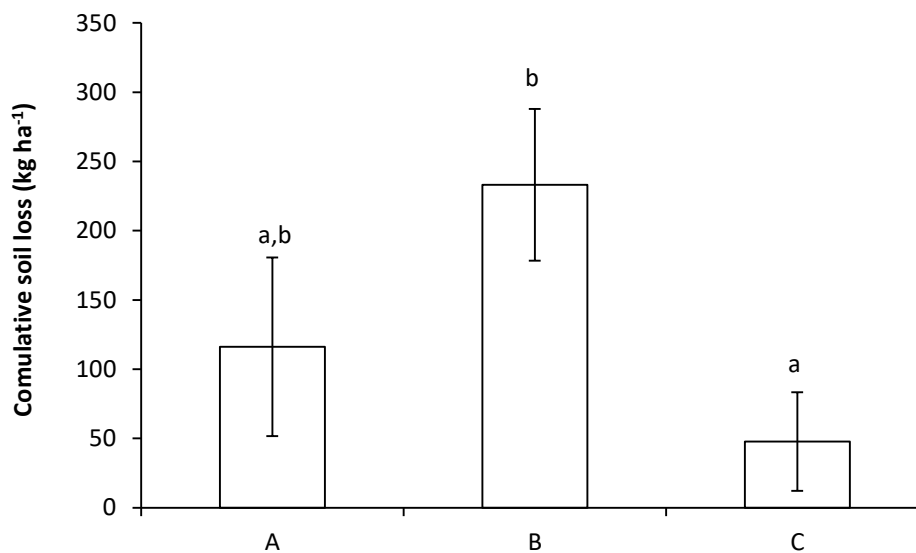


Figure 126 Comparison of cumulative soil loss (kg ha⁻¹) between locations. Vertical bars represent SE. Vertical bars represent SE. A n=8, B n=6, C n=6. Lower case letters represent homogenous groupings. A= west plots; B= central plots (depression); C= east plots.

3.3.6 Soil texture

Average clay content was $15.1 \pm 0.4\%$, silt $56.3 \pm 1.6\%$ and sand $28.6 \pm 1.9\%$, which is classified as a sandy silt loam according to the UK soil textural triangle. No significant differences were found between the treatments or locations for sand and silt. Clay was significantly different between treatments ($P < 0.05$; Figure 127). Clay percentage in the traditional method was 13% smaller compared to *L. multiflorum* and 14% compared to *L. multiflorum* & *T. Alexandrinum*. There were no differences between the traditional method and *L. multiflorum* & *V. villosa* and between *L. multiflorum* and *L. multiflorum* & *T. Alexandrinum*.

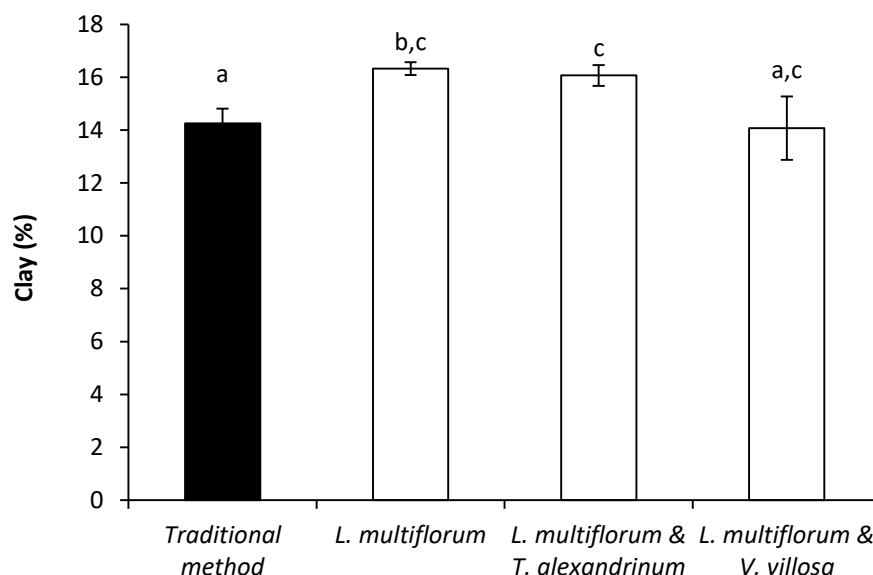


Figure 127 Comparison of clay (%) between cover crops treatments (white) and the traditional method (black). Vertical bars represent SE (n=5), letters represent homogenous groupings.

4 DISCUSSION

4.1 Survey among UK maize growers

The survey among UK maize growers had a low number of respondents for each question; this implies caution in the interpretation of the survey results; however, some conclusions can be drawn. The relevance of this project for UK maize growers is confirmed, as more than half of the respondents experienced runoff and soil erosion problems, especially in the South West of England (Figure 19). Additionally, cover cropping was the most implemented runoff and erosion mitigation strategy (Figure 20). Yet, cover cropping may not be relevant for some growers; as many respondents indicated that they cross plough post-harvest to control soil erosion or the plant wheat (Figure 20).

The principal reason to sow cover crops for growers that experience runoff and erosion problems was to reduce soil erosion, increase OM and improve soil structure (Figure 22). Interesting, 60% of growers that did not have runoff and erosion problems have also considered cover cropping mainly for OM increase and soil structure improvement (Figure 22). In both cases, only a few respondents used cover crops to enhance soil microbiology. Therefore, the potential positive impact of cover crops on soil biota is still not fully grasped and more effort needs to be done by the scientific community to sensitise the wider public.

A small number of growers indicated cover cropping as an additional income source (Figure 22). It is possible that in the UK cover crops are not an immediate form of profit but only a cost to the farmers. Cover crop seeds, additional inputs and labour probably exceed the possible incomes of a basic payment scheme. Consequently, to encourage practice adoption, there should be more financial support to the farmers that decide to invest in cover crops and in the long term health of their soil.

It was thought that one of the primary reasons behind the decision not to grow cover crops would be the potential competition with maize. However, even if

there was some concern among the maize growers, it was not the principal reason not to use cover crops. The primary motivation amongst the growers that did not experience runoff and soil erosion was the lack of clear advantages for them (Figure 23). This result emphasises the gap in the literature regarding potential benefits of cover crops in the UK and confirms that more incentives need to be provided to encourage the practice.

In reference to the 16 cover crop species suggested in the survey, only two species had more positive responses than negative among to the growers that experience runoff and soil erosion (Figure 24). Those crops were ryegrass (*Lolium* sp.) and forage rye (*S. cereale*), which in the literature are especially useful for soil erosion control. This result remarks the relevance of this project but also highlights that, perhaps, there is not enough scientific evidence proving the efficiency of other cover crops and more effort should be invested into finding new possible species.

Growers that did not have runoff and erosion problems were willing to adopt a more diverse range of cover crops (Figure 25). Among the species recommended were clover (*Trifolium* sp.), vetch (*Vicia* sp.), ryegrass (*Lolium* sp.) forage rye (*Secale cereale*), which were all used in this project, and forage rape (*Brassica napus*). All species can be incorporated into the soil to increase OM and nutrients.

UK maize growers are not sure on the best way to establish cover crops (undersowing or sowing post-harvest) (Figure 30). More research is needed on the effect of sowing dates and sowing techniques. Research should focus on the consequences of the different sowing techniques as additional soil compaction post-harvest, late establishment of the cover crop, and competition with maize and weeds.

Conclusively, the survey proved that the project is relevant to UK maize growers. It highlighted the necessity of financial investment to encourage cover crop adoption and it discovered the gap in the knowledge regarding cover crop advantages for soil health and cover crop sowing techniques.

4.2 Experiment 1

4.2.1 Cover crops

In reference to the cover crops used during Experiment 1, forage rye (*S. cereale*) had a poor establishment (visual observation) compared to Italian ryegrass (*L. multiflorum*), probably due to the adverse weather conditions post-harvest (too wet/cold) that resulted in crop height and stem density in spring (Figure 35). Maize growers with the intention of sowing this cover crop should consider the risks of crop failure. In order to eliminate the risk another cover crop species can be undersown in summer, as it is not possible to undersow rye due to the unavailability of the seed in summer. On the contrary, *L. multiflorum* under-sown with maize and *L. multiflorum* drilled post-harvest established well (visual observation) and had greater crop height and stem density than rye (Figure 35). Therefore, Italian ryegrass seems a more reliable crop than rye in case of growers prefer to sow a cover crop post-harvest.

The lack of differences in plant parameters between *L. multiflorum* drilled and broadcast in spring indicates that the more expensive drilling does not present advantages over broadcasting. However, there may have been differences in the development of the crop in autumn, after the harvest of the maize, as it was found by other studies (Brennan, Brennan and Leap, 2014).

4.2.2 Microbial biomass and microbial quotient

It was expected that *L. multiflorum* and *S. cereale* would increase MB and microbial quotient compared to not sowing cover crops due to increased plant debris, fine root turnover and roots exudates, which represent a vital energy source for soil microbiology (Bardgett et al., 2005; Eisenhauer et al., 2017). However, MB was not increased in the cover crop treatments, but it was either similar to the non-cover crop treatments or even lower (Figure 36). The reduction of MB could be related to a reduction of nutrient availability due to plant uptake, therefore, soil nutrient status could be a limiting factor to cover crop enhancement of soil health.

Microbial quotient was not affected by cover crops (Figure 37). This result indicated that the microbial communities of both cover crop treatments and non-cover crop treatments used the same level of C per unit of MB and confirms that cover crops did not have a significant impact on soil microbial growth.

The majority of studies reports an increase of MB due to cover crop legacy effects after termination and subsequent incorporation, which increases soil nutrient status that was probably a limiting factor in this experiment during cover crop growth. Jokela et al., (2009) stated an increase in MB after four years of cover cropping in maize with kura clover (*Trifolium ambiguum*), red clover (*Trifolium pratense*), italian ryegrass (*Lolium multiflorum*), and rye (*Secale cereale*) that were incorporated after termination. Schutter & Dick, (2002) reported that there was no an effect on MB during cover cropping with a mixture of *Avena sativa* and *Vicia sativa* during cover crop growth, but there was an increase of MB 2/3 months after cover crop residues incorporation.

The number of studies that have studied the effect living cover crop on soil microbial community is low. Wheatley et al., (1990) reported that MB was not affected after ten weeks of *Lolium perenne* and *Hordeum vulgare* in not fertilised soil. On the other hand, Haynes & Beare, (1997) reported that *Lolium multiflorum* and *Trifolium repens* grown in a greenhouse experiment for 12 weeks had larger MB compared to bare soil, probably because N and P were not limiting factors as basic fertilizer was applied. Similarly, Finney et al., (2017) reported an increase in MB after two months after cover crop planting, probably due to a significant increase in labile organics and a favourable soil nutrient status. Consequently, the lack of effects on MB and microbial quotient in this experiment indicate an absence of substantial C inputs by the cover crops and microbial growth could have been limited by the competition for N and P with the cover crop (Jones et al., 2018).

4.2.3 Soil chemistry

Average OM (3%) was above the minimum desirable OM level for sandy loams (1.5%) reported by Janssen, (1993), but the ratio OM to clay+ silt was below the minimum threshold (0.05) to maintain physical stability indicated by Pieri,

(1989). Organic carbon (1.4%) was below the 2% minimum threshold for maintenance of aggregate stability noted by (Greenland et al., 1975), but above the minimum limit (0.6%) to prevent the collapse of soil structure for loamy sands to sandy loams (Janssen and de Willigen, 2006). However, it was below the threshold level of SOC in the root zone (1.5–2.0%) to maintain soil physical, chemical, and biological quality (Lal, 2014). These results show that agreement on the critical limits for C and OM is still absent in the literature. Average bulk density values indicated that the soil was in general not compacted (Grossman et al., 2001).

Average values of soil pH were slightly acidic (≈ 6.5) (Figure 42), which is standard for that part of the UK (Henrys et al., 2012) and does not represent a problem for the solubility of primary soil nutrients and metals (Brady and Weil, 2016). Average saturated electrical conductivity (0.58 dS m^{-1}) indicated that the soil was not saline (Watling, 2007), which could have been otherwise a limiting factor to the growth of crops and soil biota. As expected, EC_{1-5} was different between December 2014 and March 2015 (Figure 44). The decrease could be due to many factors among which a reduction of nutrients such as Na^+ , Ca^{2+} , K^+ , and Mg^{2+} , due to the increased growth of cover crop and therefore of nutrients demand (e.g. Mylavarapu and Zinati 2009) or due to dispersion via leaching and runoff.

4.2.3.1 Carbon and organic matter

It was expected that *L. multiflorum* and *S. cereale* cover crops would increase soil OC and OM, due to fine root turnover and roots exudates (Buyer et al., 2010). In contrast, OM (Figure 38) and TOC (Figure 40) did not increase during cover crop growth, but decreased or were at the same levels as those of the treatments without cover crops. The reduction of OM and C in this experiment could be attributed to priming effect of roots exudates (rhizosphere priming effect) (Kuzyakov and Blagodatskaya, 2015). The presence of readily available C sources in a nutrient-limited environment would stimulate the degradation of stable OM and plant residues (maize stubbles) by soil microbiology, with a consequent reduction of C, as found in previous studies (e.g. Mwafulirwa et al.,

2017; Zhu et al., 2014). These results confirm that cover crops affect soil microbial community and consequently OM and C recycling.

The majority of the studies reporting an increase in OM and C used leguminous cover crops or mixtures containing cover crops with less C/N, which could reduce cover crop requirement of soil N that can be used by the microbiology instead. For example, Kong & Six (2012) found that TC increased in soil micro-aggregates (53–250 µm) at 96 and 146 days after *V. villosa* planting. Nivelle et al., (2016) reported a positive effect on TOC after five years of cover cropping using the mixture *Avena sativa*, *Phacelia tanacetifolia*, *Linum usitatissimum*, *Vicia sativa*, *Vicia faba*, and *Trifolium alexandrinum*. However, the same mixture of CC species, but with a prevalence of non-leguminous plants had an adverse effect on TOC.

Most studies reporting an increase in OM and C were also conducted over more than one growing season and looked at the effects of cover cropping after termination/incorporation (Alvarez, Steinbach and De Paepe, 2017; Nivelle et al., 2016a; Verzeaux et al., 2016b). For instance, an increase in TOC has been found in long-term studies such as after 31 years of cover cropping with *V. villosa* in a continuous cotton plantation (Mbuthia et al., 2015). Nakamoto et al., (2012) reported an increase of TOC in the first 15 cm of soil, due to six years of cover crop incorporation (*V. villosa* and *S. cereale*) in a rice-soybean rotation. Frasier et al. (2016) reported an increase in TC after three years of cover cropping with *Secale cereale* and *Vicia villosa* in a zero tillage system with *Sorghum bicolor*, no effects were observed before the third year. Additionally, Schutter & Dick, (2002) reported that there was no an effect on TOC neither during *Avena sativa* and *Vicia sativa* growth nor after 2/3 months after residues incorporation, probably due to the limited length of the study. Therefore it is possible that if this experiment would have been conducted for longer OM and TOC could have increased.

Root exudates are rapidly converted by the microbial community and limit the increase of C (Kuzyakov and Blagodatskaya, 2015). Consequently, an investigation of labile forms of C, instead of TOC and OM, could give a better

understanding of the influence of cover crops on C sequestration. For example, Jokela et al., (2009) stated that after four years of cover cropping in maize *Trifolium ambiguum*, *Trifolium pratense*, *Lolium multiflorum* and *Secale cereale* did not affect OM. However, there was an increase in active and labile forms of C.

In reference to the non-cover crop treatments, the traditional method and the cross plough method had larger OM (Figure 38) and OC (Figure 40) compared to the disc method. Most likely, the maize residues were better incorporated by the mould plough compared to the disc plough, creating a more favourable environment for the decomposition of the debris, which increased OM and OC in those treatments (Sun et al., 2014).

4.2.3.2 Soil nutrients

Cover crop treatments had lower or similar TN (Figure 39) and AP (Figure 41) compared to the treatments without cover crops. This result could be due to a priming effect, as mentioned earlier. It is also probable that *L. multiflorum* and *S. cereale* acted as catch crops, limiting nutrient leaching. Many other studies have reported a decrease in soil N due to nutrient uptake by *L. multiflorum* and *S. cereale*, e.g. Valkama et al. 2015 for a meta-study reviewing 35 studies done in northern Europe. Though, in this project, there was no reduction of nutrients in the runoff (Figure 49), even if this was probably due to the variability of the runoff collected from the different treatments (Section 4.2.4). In the long-term and after cover crop incorporation, N usually increases. After 31 years of cover cropping with *V. villosa* in a continuous cotton plantation, cover crop residues increased N content of the soil (Mbuthia et al., 2015). Nakamoto et al., (2012) reported an increase of TN in the first 15 cm of soil due to six years of cover crop incorporation (*V. villosa* and *S. cereale*) in a rice-soybean rotation. Frasier et al. 2016 reported an increase in TN after three years of cover cropping with *Secale cereale* and *Vicia villosa* in a zero tillage system with *Sorghum bicolor* at 0.06-0.12 m depth, thanks to root exudations.

4.2.4 Runoff and soil loss

The presence of cover crops did not significantly affect runoff (Figure 48), soil loss (Figure 52), or nutrients in the runoff (Figure 49; Figure 50; Figure 51). This result is probably due to the high variability within treatments. It is feasible that some plots were located in areas with low moisture storage capacity due to the presence of a shallow water table or less permeable layers that increased the occurrence of runoff and, therefore, soil loss.

Many papers have recorded high variability in runoff and soil loss within the same treatment, despite replication (up to 40 in Gómez *et al.*, 2001), refer to Rüttimann *et al.* (1995) and Hjermfelt & Burwell, (1984) for other examples. The variability can be attributed to i) the extreme complexity and the scale-dependency of the erosion process, ii) the natural variability of the soil environment and iii) inevitable disturbance caused by the design of the erosion plots. Nevertheless, many studies, have reported a significant decrease in runoff and soil loss by cover crops (Dabney *et al.* 2010; Delgado *et al.* 2007), especially by grass species with fine-branched roots (De Baets *et al.*, 2011b). For example, there was a 13% reduction in runoff and soil loss under rye (*Secale cereale*) in a conventional maize system (Martin & Cassel, 1992). Additionally, Laloy and Biielders, (2010) reported a reduction 94% of soil erosion in maize under rye (*Secale cereale*) and ryegrass (*Lolium multiflorum*) compared to post-harvest maize stubbles.

4.3 Experiment 2

4.3.1 Cover crops and maize yield

All cover crop treatments established well by November 2015 despite broadcasting the cover crops by hand and on average 80% of soil surface was covered by the cover crops (Figure 55). Contrary to expectations, cover crop mixtures did not have an increased dry biomass and canopy cover compared to *L. multiflorum*, regardless of the increased seed density. An increase was found by other studies (e.g. Finney *et al.*, 2016). This result demonstrates that *L. multiflorum* seeded at 56 kg ha⁻¹ is an extremely competitive species, able to

provide adequate canopy cover alone and that probably the plots were seeded at capacity. Maize yield was not affected by the presence of under-sown cover crops, despite many studies reporting an adverse effect (Iqbal et al., 2015; Pantoja et al., 2015), probably because of lack of competition for water.

4.3.2 Soil biology

4.3.2.1 Microbial biomass and microbial quotient

Microbial biomass (Figure 57) and microbial quotient (Figure 90) were not altered by the presence of cover crops, probably because cover crops were grown only for one season. As stated earlier, the majority of studies which reported an increase in MB and microbial quotient were conducted over multiple growing seasons and focused only at the legacy effects of the cover crops (e.g. Dinesh et al., 2006; Sainju et al., 2007). However, differences in MB were found between sampling dates (Figure 58); MB reduced from November 2015 to March 2016, probably due to colder weather conditions in March that restricted microbial growth.

Additionally, it was supposed that planting a mixture of grass-legumes would increase MB and microbial quotient compared the use of grass alone, due to an increased plant diversity and plant biomass (Zak et al., 2003). Yet, no evidence of this was found (Figure 59), probably due to the fact that the cover crop mixtures (*L. multiflorum* & *T. alexandrinum* and *L. multiflorum* & *V. villosa*) had the same biomass as the pure stand of *L. multiflorum* (Figure 53) as it was reported by Zak et al., (2003). Zak et al., (2003) investigated the effects of plant diversity on microbial parameters such as MB, FB and respiration. After seven years of plant growth the changes in MB, FB and respiration were attributed to the increased plant biomass rather than the increased plant diversity. However, N mineralisation was influenced by plant diversity.

Finney et al., (2016), asserts that when a mixture contains crop with complementary plant traits (phenology or N strategy acquisition) the effect of the crops on soil microbiology increases; whereas if the crops in the mix have similar plant traits no enhancement is found. In support of this theory, Wortman

et al. 2013 reported that two mixtures (*Vicia villosa* & *Fagopyrum sagittatum* and *Vicia villosa* & *Sinapis alba*) had larger MB compared to a mixture of 8 cover crops in a sunflower–soybean–corn rotation after three years of cover cropping. This result was probably due to the lack of complementary plant traits in the mix. The crop used in the mixture in this project had complementary plant traits and therefore should have had a more significant effect than the single species. However, at the end of the experimentation, it was visually noticed that *L. multiflorum* was prevailing in the mixtures as well as in the pure stands, perhaps limiting the potential benefits of the mixtures. Therefore, species completion could limit the potential benefits of a mixture of cover crops.

The effects of compaction created by the harvester on MB and the combined effect of compaction and cover cropping was also investigated by measuring MB both in and outside the wheel tracks in November 2015. To our knowledge, it is the first time that the effects of first time compaction and cover cropping on soil microbiology are simultaneously investigated in maize in the UK.

There is lack of agreement in the literature on effects of compaction on soil microbiology (Beylich et al., 2010). In some cases, MB could increase after a light compaction because the reduction of macro-pores could limit microbial predators (Jensen et al., 1996; Pengthamkeerati, Motavalli and Kremer, 2011). However, it decreases after a certain threshold, after which, gas and water movement are limited (Jensen et al., 1996; Pupin, Freddi and Nahas, 2009; Weisskopf et al., 2010). This research is relevant since large parts of fields are affected by wheel tracks caused by the harvest. Cover crops did not have a significant effect on MB of the soil collected in the wheel tracks (Figure 60Figure 59), and there was no difference in MB between soil collected in the wheel tracks and outside the wheel tracks (Figure 60). Consequently, we can assume that in this experiment compaction was moderate (Pengthamkeerati, Motavalli and Kremer, 2011) or that the microbial community was resilient (e.g. Shestak & Busse, 2005).

4.3.2.1 Multiple substrates induced respiration and metabolic quotient

The functional profile of the microbial community was not altered by the cover crops (Figure 81 Comparison of microbial community functional profile between the cover crop treatments and the traditional method. PCA score plots of the means, PC 1 and 2 (Treatments $n=5 \pm \text{SE}$; Sampling date $n=20 \pm \text{SE}$). Samples collected in November 2015 (white) and samples collected in March 2016 (black). Letters represent homogenous groupings on PC 1 (Sampling date). Traditional method= no cover crops, mouldboard. Figure 81). The absence of differences in the first three PC of the MSIR profile could indicate that the microbial communities of the different treatments were equally adapted to use the substrates provided. Therefore, we can assume that they could offer similar ecosystems services related for example to C sequestration and nutrient cycling (Wakelin et al., 2008).

As proven by many studies on large and small scales the functional profile of the microbial community is driven by C and pH. For example, a previous study by Wakelin et al., (2008) on Australian soils found that pH was the principal driver of the microbial functional profile of agricultural soils. Similarly, soil pH and OC were the discriminant factors in a pan-European study that covered more than 81 sites (Creamer et al., 2016). In this project, treatments did not affect pH (Figure 105) and TOC (Figure 96); consequently, the functional profile of the microbial community was similar across the treatments.

Possibly, significant effects of the cover crops could become evident after cover crop incorporation. For example, Nakamoto et al., (2012) reported an increase in microbial respiration (glucose) under *S. cereale* compared to under *V. villosa* and bare fallow three weeks after crops incorporation. This result was probably caused by the increased amount of C and nutrients in the soil after the cover crop incorporation that favoured the development of a microbial community able to use those resources quickly. Similarly, Nakamoto & Tsukamoto, (2006) study on bacterial and fungal substrate-induced respiration (glucose) reported that the use of *Trifolium repens* enhanced bacterial and fungal respiration compared to

bare fallow. This result was attributed to an increase of microbial and fungal biomass. Schutter & Dick, (2002) reported there was no an effect on soil respiration during cover cropping with a mixture of *Avena sativa* and *Vicia sativa* but that the functional profile of the cover crops was significantly different compared to bare fallow after 2/3 months after residues incorporation. They also reported a season effect as found in this project (Figure 81).

The compaction created by the harvester did not affect MSIR. Consequently, compaction altered the composition of the microbial community (phenotypic profile; Figure 64) but did not change its functionality (Figure 83). This result is probably due to the functional redundancy of the microbiota that allows different communities to perform similar functions (Nannipieri et al., 2017; Setälä, Berg and Jones, 2005). However, there is evidence that some ecosystem function such as N and C cycling are modified by phenotypic differences, even if it is not clear at what level of diversity functionality is impaired (Coleman and Whitman, 2005; Hanson et al., 2008). Consequently, a more detailed investigation on potential nitrification, for example, could help clarifying how cover crop induced changes in the microbial community affect specific ecosystem services such as nutrient cycling.

Metabolic quotient was not affected by one season of cover crop implementation (Figure 89). Therefore, the presence of CC did not change the metabolic state of the soil microbial community. Authors have interpreted an increase in qCO_2 as an indicator of stress and decreased in substrate use efficiency as the microbial community need to use more resource to principal a certain level of biomass, resulting in an enhanced respiration (Anderson and Domsch, 1993; Insam and Haselwandter, 1989). An increment in fungi over bacterial is usually associated with a decrease in qCO_2 indicating a larger substrate efficiency use and more mature and stable phase of the microbial community evolution (Sakamoto and Oba, 1994). However, even if there was an increase in F/B (Figure 75) the qCO_2 was not altered, most likely because bacteria were still dominating the microbial community.

4.3.2.2 Phospholipid fatty acid analysis

Cover crop effect on the microbial phenotypic profile was limited (Figure 61), as the profile was only affected by *L. multiflorum* & *T. Alexandrinum*. However, there was a noticeable divergence of the cover crop treatments in the PCA score plot from November 2015 to March 2016 (Figure 62), which was missing in the traditional method. This result supports the hypothesis of a microbial community shift caused by the presence of cover crops. Nevertheless, further research is needed. Furthermore, since the mixture *L. multiflorum* & *T. alexandrinum* altered the phenotypic profile, additional work is required to investigate the effect of other cover crop species. It is possible that if the experiment was conducted for a more extended time and if data was collected after cover crop incorporation, cover crop effects would be evident as occurred in other studies (e.g. Schutter et al., 2001).

The compaction created by the harvest affected the phenotypic profile of the microbial community on PC1 (Figure 64). This result indicates that the microbial community composition changed after the compaction, species that are more competitive in a compacted environment, for example with less oxygen, were dominant (Linn and Doran 1984). Cover crops did not affect the phenotypic profile of the microbial community of compacted areas (Figure 63) possibly because the majority of plants were killed by the compaction (visual observation).

4.3.2.1 Microbial markers, fungal biomass and glomalin-related soil proteins

In regards to the relative abundance of the microbial markers, *L. multiflorum* had a similar relative abundance of bacteria compared to the traditional method, while the mixtures grass-legume had a lower relative abundance of bacteria (Figure 65). It seems that, even if bacteria dominated in all treatments, the leguminous plants favoured the development of fungi, rather than bacteria, resulting in a lower relative abundance of bacteria. The relative abundance of fungi revealed that all cover crop treatments including *L. multiflorum* had a larger relative abundance of fungi compared to the traditional method. This

result demonstrates a shift in the structure of microbial community in the cover crop treatments. It is possible that bacterial growth was limited by the competition with the cover crops for nutrients. On the other hand, fungi could have been favoured by their ability to explore the soil matrix in search of nutrients and by their stoichiometry which has a higher C/N compared to bacteria (Strickland and Rousk, 2010). However, there were no significant differences in fungal biomass (Figure 86) or fungal/bacterial biomass (Figure 87) between the cover crop treatment and the control. This result was probably due to the high variability (SD) between the treatments caused by the lack of repeated measures. Additionally, discrepancies between fungal biomass estimated with ergosterol and fungi18:2 ω 6,9 have been reported before in the literature (Högberg, 2006). Therefore, possibly, plant residues that also contain 18:2 ω 6,9 contaminated soil samples (Olsson, 1999). However, this effect was minimal since soil samples were sieved to eliminate roots and other plant parts.

Cover crop effect could be more evident after residues incorporation. Many studies report an increment in fungi after cover cropping due to residues which is more available to fungi than to bacteria (Lucas, Angelo and Williams, 2014). For example Nakamoto et al., 2012 reported an increase of F/B activity due to cover crop incorporation, after six years of cover cropping with *V. villosa* and *S. cereale* in a rice-soybean rotation, and after three years under *Avena strigose* and *V. villosa* in spring wheat cultivation. The incorporation of *V. villosa* was found to enhance fungal biomass (ergosterol) from five days after residue incorporation, due to the cellulosic nature of residues. Kataoka et al., (2017) found an increase in fungal biomass (ergosterol) due to *V. villosa* residues incorporation. Similarly, Lucas et al., 2014 reported an increase in F/B markers after the incorporation of *V. villosa* in a pot experiment with three different soil types. Calderón et al. 2016 reported an increase in fungal markers (18:1 ω 9c, 18:2 ω 6c and 18:3 ω 6c) after cover crop termination (one season; *Avena sativa*, *Pisum sativum*, *Linum usitatissimum*, *Brassica napus*) compared to bare soil in no-till wheat.

Other studies found a reduction of F/B over time due to the incorporation of high-quality cover crop residues (low C/N) (e.g. Brennan & Acosta-Martinez 2017, Frasier et al. 2016). Another factor that could affect the F/B is the nature of the mycorrhizal association. If AMF are abundant a change in F/B markers or biomass could not be evident. For example, Murugan et al., (2014) reported that a two year's old modified grassland (*Lolium perenne*, *Phleum pratense*, *Poa pratensis* and *Trifolium repens*) had less fungal biomass (ergosterol) compared to maize cultivation and less F/B biomass. However, the grassland had larger fungal C compared to microbial C. This enhancement was attributed to the dominance of AMF fungi, which do not contain ergosterol (Olsson et al., 2003), and consequently are not counted in the fungal biomass. Moreover, the fungal marker 18:2 ω 6, 9 is an indicator only of saprotrophic fungi and ectomycorrhizae (Frostegård & Bååth, 1996). Therefore, AMF markers are not counted in the F/B.

The relative abundance of the AMF marker 16:1 ω 5, in this experiment, was significantly lower in the mixture *L. multiflorum* & *T. alexandrinum* compared to *L. multiflorum* alone and compared to the traditional method without cover crops (Figure 78). The presence of leguminous plants seemed to have favoured saprophytic fungi over AM fungi, at least in the case of the mixture *L. multiflorum* & *T. alexandrinum*. A possible explanation is that mycorrhizal associations were not advantageous for the plants due to sufficient level of N and P or that the host plant (*T. alexandrinum*.) was not compatible with the AMF species present (Torrecillas, Alguacil and Roldán, 2012; Vandenkoornhuyse et al., 2002). However, the accuracy in the determination of AMF presence could have been altered by the presence of Gram-negative bacteria that also contain the marker 16:1 ω 5 (Frostegård, Tunlid and Bååth, 2011). Additionally, *L. multiflorum* & *V. villosa* had a similar relative abundance of AMF compared to *L. multiflorum* alone and compared to the traditional method. Similarly, Finney et al., (2017) reported that non-AM fungi were correlated with hairy vetch (*Vicia villosa*) growth.

The treatment *L. multiflorum* & *V. villosa* reduced the relative abundance of Gram-positive bacteria (Figure 67), while *L. multiflorum* & *T. alexandrinum* reduced the relative abundance of Gram-negative bacteria (Figure 69). These differences are probably due to the different selection continuum of Gram-positive and Gram-negative bacteria. Gram-positive bacteria are usually oligotrophs (*K*-selection continuum), while Gram-negative are copiotrophs (*r*-selection continuum) (Finney et al., 2017). Possibly, the more extensive root system of *V. villosa* compared to *T. alexandrinum* increased the presence of root exudates and fine root turnover in *L. multiflorum* & *V. villosa* compared to *L. multiflorum* & *T. alexandrinum*. These labile forms of C are preferred by Gram-negative bacteria (Kramer and Gleixner, 2008). Therefore, the decrease of Gram-negatives could be attributed to an increased relative abundance of Gram-negative bacteria. However, more research is needed to confirm this suppositions.

Average glomalin across the experiment was in the range (50-350 ppm) of other studies (Balota et al., 2014; García-González et al., 2016a; Redmile-Gordon et al., 2013). However, no significant differences were found (Figure 88), probably because of the relative abundance of the AMF marker 16:1Ω5 was similar across the treatments (Figure 78). Many studies have proven the relationship between glomalin related proteins and the abundance of AMF (García-González et al., 2016a). Balota et al., (2014) reported an increase in glomalin in the first 5 cm of soil in a conventional soybean-maize system after 23 years of cover cropping with *Avena strigose*, *Triticum aestivum*, and *Vicia villosa* and with *Avena strigose*, and *Vicia villosa* in a no-till soybean-maize system due to enhanced AMF. Similarly, García-González et al., 2016 reported an increase of glomalin after six years of cover cropping with *Hordeum vulgare* in a sunflower-maize system.

4.3.3 Soil chemistry

Average OM (3%), TOC (1.3%), soil pH (6.4) and saturated electrical conductivity (0.52 dS m⁻¹) were similar to Experiment 1. Cover crops did not affect any soil property (Table 19). Possibly, as stated before, if the experiment

was conducted for more extended time OM and C could have increased, and maybe the synergic effect of using cover crop mixtures instead of single species would have been evident as in other studies (Muzangwa, Chiduza and Muchaonyerwa, 2015). Additionally, micro-topography may have masked treatment effects as the location of the plots significantly changed soil properties. Soil pH was lower in A compared to B and C. Probably it was altered by the accumulation of cations in the depression that was location B, confirming topography investigations. Other studies have reported an increase in soil pH in soil depressions (e. g. Baltensweiler et al., 2017; Ofori, Atakora and Antwi, 2013). The location had a consistent and significant effect also on OM, TOC, TN and AP (Table 19). OM (Figure 92), TOC (Figure 98), and TN (Figure 94) were the lowest in location B, and the greatest in location A. Available P had an opposite trend being the lowest in location A and the most significant in location B (Figure 101). This tendency was due, most likely, to the increased solubility of P at a slightly higher pH compared to A and C. The decrease of TN, TOC and OM in B could be attributed to the increased of microbial activity due to larger pH (Kemmitt et al., 2006). The differences in soil chemistry between the three areas could have contributed to the limited effects on soil microbiology.

4.3.4 Aggregate stability

Cover crops did not affect aggregate stability (Figure 108). This result contradicts other studies that report an increase of aggregate stability due to cover cropping. For example, Haynes & Francis, (1993) stated that growing *L. multiflorum* increased aggregate stability (estimate as mean weight diameter) in a short-term (4 months) study compared to maize. Additionally, Haynes & Beare, (1997) reported that in a 12 weeks greenhouse experiment *Lolium multiflorum* and *Trifolium repens* had larger increased aggregate stability (estimate as mean dry weight diameter) compare to the traditional practice. Probably in this short-term studies cover crops had a significant input of C, which did not occur in this project or in Schutter & Dick, (2002). Additionally, studies typically report an increase of stable aggregates after cover crop termination (Hermawan and Bomke, 1997; García-González et al., 2016). For example, in a

pot experiment, the incorporation of *L. multiflorum* residues significantly increased water-stable aggregates compared to no adding crop residues (Oyedele et al., 1999). Miller & Dick, (1995) found after five years of cover cropping with residue incorporation of *Trifolium pratense*, a 33% increase of macro-aggregate (>0.25 mm). Nakamoto et al., (2012) reported an increase of aggregate stability (estimate as mean dry weight diameter) in the first 7.5 cm of soil, due to six years of *V. villosa* and *S. cereale* incorporation in a rice-soybean rotation. García-González et al., (2016b) found an increase of water-stable aggregates after six years of cover cropping with *Hordeum vulgare* and *Vicia villosa* in a sunflower-maize system 54 days after planting the main crop.

4.3.5 Runoff and soil loss

Although, average runoff and soil loss were larger in the plots without cover crop compared the cover crop treatments, the presence of cover crop did not statistically affect overall runoff production (Figure 120) or soil loss (Figure 121). The lack of significant differences was likely due to high deviation (SE) within the treatments caused by the spatial heterogeneity of the field. Differences in field micro-topography (Figure 1) led to a differentiation of the runoff flow direction (Table 11), which became the principal factor that contributed to the variation between in runoff and soil loss recorded for the different erosion plots. Runoff and soil loss collected in area C were significantly less than in area B (Figure 124). This result was in large part due to the micro-topography of the field, which probably facilitated the convergence of sub-surface soil moisture in the depression, area B, decreasing water infiltration, increasing runoff velocity and soil erosion. Additionally, the presence of subsidiary slopes inside the plots in area A and C led to an underestimation of the runoff and soil loss collected in those areas, adding to the variation within the treatments.

No differences were found between cover crop canopy cover (Figure 54) and biomass (Figure 53), this probably contributed to the absence of differences in runoff and soil loss between *L. multiflorum* and the mixtures of *L. multiflorum* with legumes. Therefore, if cover cropping is adopted for solely erosion control *L.*

multiflorum alone is a better option compared to using a mixture of grass and legumes.

Despite the lack of significant effects of the cover crops on runoff and soil erosion, there was a clear trend towards the reduction of runoff and soil erosion. Other studies found a reduction of runoff and soil loss in maize due to cover cropping, e.g. (Martin & Cassel, 1992; Laloy and Bielders, 2010). Therefore, significant effects could be found if an increased number of replicates and a longer period of implementation is used.

4.4 Project limitations

Experiment 1 was fitted into an ongoing trial where plot design had taken into account the ease of field management; because of this, plots had not been randomly allocated in the field area. This allocation gave rise to uncertainty in the interpretation of experimental results due to possible underlying changes in soil properties.

For Experiment 2, joined erosion plots shared a common central border, in order to reduce the land take, locate the plots in a uniform area and also minimise the disruption of farming operations. However, the plot size (15x1.5m) may have impacted the behaviour of soil microbiology. It is feasible that AM fungi, while symbiotic with a cover crop, had the majority of their hyphae located in plots without cover crops because of the proximity of the plot areas. Also, the harvester was not able to pass between them because the plots were so close one another. Consequently, the experimental plots had to be removed and reinstalled, interrupting the monitoring programme. Also, the harvesting operations resulted in wheel tracks within the plot areas, which probably had an impact on the runoff and soil loss results of subsequent erosion events. However, the effect of the wheel tracks could be considered to be consistent across all treatments.

After the maize harvest, it became clear that the experimental plots were located in an area where the slope was not as homogenous as initially thought. The central plots were in a slight depression efficiently creating three areas of

plots with similar micro-topography. If the maximum slope, only 3-4°, had been more prominent any secondary slope in the experimental plots probably would have had less of an impact on results. However, it would seem that the micro-topography may have masked any treatment effects. Furthermore, since the experimental design did not take account of these areas, treatment replications were not evenly distributed.

Collecting sampling within the erosion plot areas for microbiology and soil chemistry created areas of disturbance that could have affected runoff and soil loss collection. However, care was taken to limit the disruption, and an equal amount of disturbance was created in each plot area.

It is uncertain how much edge effect impacted on experimental results. It is probable that broader erosion plot areas would have been better at reducing edge effect. However, for the reasons stated above, larger plot areas were not practical at this site. The size of the experimental plots also made it difficult to accurately locate them once the soffit boards were removed and the cover crops incorporated. Subsequently, it was not possible to investigate the effects of cover crop incorporation.

Problems were encountered during the setup and realisation of the erosion plots. In Experiment 1 tank stability was compromised by a shallow water table, which was resolved by the installation of ground anchors. This issue disrupted the collection of the runoff data. During both experiments, experimental data was lost because of damage caused by deer breaking the cables connecting the sensors and due to loss of power to the weather station. This unexpected event prevented the use of the linear level sensors in the experiment, which could have been used to correlate runoff production to a single rainfall event.

5 Conclusion

It was hypothesised that planting cover crops during maize production would affect soil microbial community compared to various conventional post-harvest methods that do not imply cover cropping. Over the course of the project, the effect of the cover crops on soil microbiology was limited. Soil microbial community shifted towards more abundant fungi in the cover crop treatments. However, microbial and fungal biomasses were not altered by one season of cover cropping. Therefore, using *L. multiflorum* and the cover crop mixtures *L. multiflorum* & *T. alexandrinum* and *L. multiflorum* & *V. villosa* has only a partial effect on soil microbial community in the short term.

Additionally, it was hypothesised that the cover crop mixtures would have a more significant effect on soil microbiology than *L. multiflorum* alone. The only treatment that affected the phenotypic profile of the microbial community was *L. multiflorum* & *T. alexandrinum*, yet all cover crop treatments affected the relative abundance of fungi. Consequently, in this study, the cover crop mixtures did not have a clear advantage compared to *L. multiflorum* alone.

It is clear from the literature that the magnitude of the effects on the microbial community will depend on the nutrient status of the soil and will be more evident after residues incorporation. Mixtures of grasses and legumes could have a lower nutrient requirement in the long-term compared to pure grass; this could leave more nutrients available to the microbial community. Additionally, leguminous cover crops could release N in the soil after incorporation. Therefore, legumes-grasses mixture could have a greater effect on the microbial community in the long term. However, an addition of organic or synthetic fertiliser could compensate for the uptake of N and P by grass cover crops.

In regards to cover crop effect on SH, cover crops could have an enhancing effect, but the short-term effect were limited. An increase in soil fungi could promote SH as fungal-dominated communities have an enriched microbial-derived OM and are considered more stable (Six et al., 2006). Nevertheless, in

this research, the soil functional profile showed that even with a differentiation of the microbial community composition, the ability of the microbial community to process different substrates was not altered. Consequently, in the short term, the microbial community supported by the cover crops provided similar ecosystems services, related for example to C sequestration and nutrient cycling (Wakelin et al., 2008), to the microbial community of the traditional post-harvest methods (no cover crops). Additionally, OC, OM, aggregate stability and glomalin were not altered due to the short period of implementation and the absence of residues incorporation. Consequently, it is necessary to implement soil conservation practices over a more extended term to see changes in SH, which may take more than four years to be evident (Jokela et al., 2009).

The microbial community that can develop after residues incorporation could potentially have different phenotypic and functional profile from the community present during cover crop growth. Therefore, more investigation is required to understand how the community evolves over the course of the cover crop growing cycle and how the incorporation of residues, due to tillage, will affect the previously enhance the fungal community. DNA analysis could help identify the species that are affected and future research should be conducted for longer than one growing season to investigate the long-term effects. Cover crops could also have an impact on soil macro and mesofauna. Consequently, key soil organisms like earthworms, nematodes and beetles should also be investigated to have a complete overview of the effects on SH.

It was hypothesised that planting cover crops during maize production would reduce runoff and control soil erosion compared to various conventional post-harvest methods that do not imply cover cropping. The presence of a cover crop did not statistically affect overall runoff production or soil loss, due to the spatial heterogeneity of the field. Although, trends indicated a potential reduction of runoff and soil loss due to cover crops. Regarding the use of grass-legume mixtures, *L. multiflorum* & *V. villosa* and *L. multiflorum* & *T. alexandrinum* did not have a clear advantage on soil erosion control over the use of *L. multiflorum* alone.

Future research should continue to investigate the effect of grasses on runoff and erosion control. It is recommended that a steeper slope ($>40^\circ$) should be examined to limit the impact of micro-topography. Soil micro-topography should be detailed assessed as support for explaining experimental results. Furthermore, investigation on seeding rates, time of sowing and the effects of above and below-ground plant traits need to be explored in more details.

In conclusion, due to the absence of a negative impact on maize yield and the encouraging results on runoff, soil erosion and soil fungi, cover crops could potentially improve the sustainability of maize, even if short-term effects are limited. Further long-term investigation should be conducted to uncover the full potential of cover cropping with maize in the UK.

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APPENDICES

Appendix A Maize Growers Association Survey

PARTICIPANT CONSENT FORM

The following questionnaire has been developed by Cranfield University to inform research on erosion and runoff control by maize growers. The data collected in this study will be used to address the current understanding and future directions of two PhD projects. Data will be used for research purposes only at Cranfield University as part of these projects. All data collected will be stored in accordance with the UK Data Protection Act (1998) in a secure folder only accessible by Cranfield University staff. All information will be treated with the strictest confidence and questionnaire data will remain anonymous. Research findings may form the basis of scholarly publication/ academic presentation, but no participating individuals/organisations will be named. Participants are free to withdraw their data without explanation by emailing a.mancini@cranfield.ac.uk or alexandra.cooke@cranfield.ac.uk at any point. If you have any questions about the research, or how your data will be used please do not hesitate to contact Agnese Mancini via email (a.mancini@cranfield.ac.uk) or Alexandra Cooke (alexandra.cooke@cranfield.ac.uk). By continuing to the next screen you confirm that you have read and fully understand the information provided above, and therefore give your consent to taking part in this research.

THE PROJECTS

Cover crops can be under sown with the maize to provide ground cover once the maize has been harvested. Depending on the type of crop, they can add soil organic matter, increase soil nitrogen, decrease soil erosion and runoff production, limit nutrients loss and increase SH. Agnese's project is looking at selecting most effective cover crops to reduce soil erosion and runoff together with increase soil quality and health. Filter socks are tubular meshes that are typically filled with compost and/or bark and are widely used in the US on construction sites to control sediment and nutrient losses. Alexandra's project is to identify whether these are suitable for use in an agricultural context, and if not how they can be adapted. Alexandra is developing 'filter socks' to be used in field as measures to control sediment,

erosion, runoff and phosphorus losses under different climate change scenarios.

1. As a maize grower, have you ever had problems with soil erosion and/or runoff?

- ☐ Yes (1)
- ☐ No (3)

Answer If As a maize grower, have you ever had problems with soil erosion and/or runoff? Please, tick all... Yes Is Selected

2. How did you deal with the problem?

- ☐ I have not done anything to deal with the erosion and/or runoff (1)
- ☐ I have not done anything to deal with the erosion and/or runoff but I am currently in consultation with/ have previously consulted individuals to find a solution (2)
- ☐ I have implemented erosion and/or runoff control (3)

Answer If As a maize grower, have you ever had problems with soil erosion and/or runoff? Please, tick all... Yes Is Selected

3. Which of the following erosion and/or runoff control technologies did you implement? Please, tick all that apply:

- ☐ Changing the ploughing regime (1)
- ☐ Using cover crops (2)
- ☐ Using manures (3)
- ☐ Contour cropping (4)
- ☐ Grassed waterways (5)
- ☐ Creating land terraces (6)
- ☐ Creating contour bunds (7)
- ☐ Buffer Strips (8)
- ☐ Silt fences (9)
- ☐ Filter berms (10)
- ☐ None (11)
- ☐ Other, please specify: (12) _____

4. According to your main considerations regarding erosion and runoff control technologies please rank the following by dragging them:

- _____ Off-site impacts (1)
- _____ Impacts on the crop (2)
- _____ Costs of the control technology (3)
- _____ Ecological impacts on site (4)
- _____ Longevity of the technology (5)

5. Are you considering or have you ever considered under sowing cover crops with maize?

- ☐ Yes (1)
- ☐ No (2)

Answer If Are you considering or have you ever considered under sowing cover crops with maize? Yes Is Selected

6. Why would you consider to under sow cover crops? Please, tick all that apply:

- ☐ Decrease soil erosion and/or runoff (1)
- ☐ Meet the Ecological Focus Area (EFA) for greening within the Basic Payment Scheme (2)
- ☐ Additional income source (3)
- ☐ Increase soil organic matter (4)
- ☐ Decrease herbicide use (5)
- ☐ Other, please specify: (6) _____

Answer If Are you considering or/have you ever considered under sowing cover crops with maize? No Is Selected

7. Why would you not considered under sowing cover crops?

- ☐ I am afraid cover crops will negatively affect maize yield (1)
- ☐ I have never come across this practice before (2)
- ☐ I do not see any advantage for me (3)
- ☐ Other, please specify: (4) _____

If As a maize grower, have you ever had problems with soil erosion and/or runoff? Please, tick all... Yes Is Selected

8. **Cover crops have multiple purposes, which allow them to address different agricultural applications. According to your priorities, please rank the following by dragging them:**

- _____ Cover crop should decrease erosion and/or runoff (1)
- _____ Cover crops should increase nutrient retention (2)
- _____ Cover crops should increase soil nitrogen content (3)
- _____ Cover crop should have minimum interference with the maize (4)
- _____ Cover crops should provide seeds for wild birds (5)
- _____ Cover crops should provide sources of pollen and nectar (6)
- _____ Cover crops should provide an additional economic benefit (7)

9. Different plants can be used as cover crops. Please express your willingness to under sow the following crops in the first column and justify your choice in the second column:

							Non suitabl		
	Ye	N		Valuabl	Green	Benef	e for	Competiti	
	s	(2	Uncertai	e yield	manur	it for	the	on with	Othe
	(1)	)	n (3)	(1)	e (2)	e (3)	(4)	maize (5)	r (6)
Vetch (1)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Forage Rye (2)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Ryegrass (3)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cocksfoot (4)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Barley (5)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Phacelia (6)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Oats (7)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Triticale (8)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Linseed (9)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Millet (10)	☐	☐	☐	☐	☐	☐	☐	☐	☐

Fodder									
radish	☐	☐	☐	☐	☐	☐	☐	☐	☐
(11)									
Bird's									
foot	☐	☐	☐	☐	☐	☐	☐	☐	☐
trefoil									
(12)									
Sainfoin	☐	☐	☐	☐	☐	☐	☐	☐	☐
(13)									
Musk									
mallow	☐	☐	☐	☐	☐	☐	☐	☐	☐
(14)									
Winter									
turnip	☐	☐	☐	☐	☐	☐	☐	☐	☐
rape									
(15)									
Alfalfa	☐	☐	☐	☐	☐	☐	☐	☐	☐
(16)									
Clover	☐	☐	☐	☐	☐	☐	☐	☐	☐
(17)									
Mustard	☐	☐	☐	☐	☐	☐	☐	☐	☐
(18)									
Forage									
rape	☐	☐	☐	☐	☐	☐	☐	☐	☐
(19)									
Other,									
please	☐	☐	☐	☐	☐	☐	☐	☐	☐
specify:									
(20)									
Other,									
please	☐	☐	☐	☐	☐	☐	☐	☐	☐
specify:									

(21)

10. Please select from the drop-down list the region of the county you predominately farm?

- ☐ East of England (1)
- ☐ East Midlands (2)
- ☐ London (3)
- ☐ North East England (4)
- ☐ North West England (5)
- ☐ South East England (6)
- ☐ South West England (7)
- ☐ West Midlands (8)
- ☐ Wales (9)
- ☐ Scotland (10)
- ☐ Northern Ireland (11)

Answer If Please select from the drop-down list the region of the county you are from: Scotland Is Not Selected And Please select from the drop-down list the region of the county you are from: Northern Ireland Is Not Selected And Please select from the drop-down list the region of the county you are from: Wales Is Not Selected

11. Are you in a Catchment Sensitive Farming (CSF) area? If unknown, please follow the link:

- ☐ Yes (1)
- ☐ No (2)

Appendix B CONSOWA conference paper

INTERACTION BETWEEN COVER CROPS AND SOIL MICROBIOLOGY DURING MAIZE CULTIVATION

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Introduction

Conventional maize-after-maize systems in the UK are frequently associated with on-site soil degradation and off-site environmental pollution (Boardman, 2013). This is due to a combination of factors including late autumn harvest (often in wet soil conditions) and post-harvest management that leaves minimal soil protection from stubble over autumn and winter. Typically these actions lead to soil compaction, surface runoff and soil erosion (Posthumus et al., 2011). Cover crops (CCs) can be used as a soil erosion control measure (Langdale et al., 1991) and potentially offer additional soil health benefits through their interaction with the soil microbial community and by influencing soil physical and chemical processes (Brennan and Boyd, 2012). This research aimed to study the effect of CCs (under-sown with maize) on the soil microbial community, and infer whether these changes reduced soil erodibility and improved sustainable maize cultivation.

Methods

The field trial ran from August 2015 to March 2016 in the Lugg sub-catchment (UK) (OS grid references: 344505 E, 257646 N). Annual average rainfall for the area is 794mm yr⁻¹ (Met Office, 2016). The site was a sandy loam soil, and had field slopes of 3° to 4°. Experimental variables comprised four treatments: i) Italian ryegrass (*Lolium multiflorum*), ii) Italian ryegrass and hairy vetch (*Vicia villosa*), iii) Italian ryegrass and berseem clover (*Trifolium alexandrinum*), iv) No CCs (post-harvest wintered maize stubbles) replicated five times and arranged randomly across the slope. Microbiological analyses comprised microbial biomass, phospholipids fatty acid analysis (PLFA) and multiple substrates induced respiration. Microbial biomass was determined using the fumigation extraction method (Anderson and Domsch, 1978) using a K_{ec} of 0.45 (Vance et al., 1987). The phenotypic profile of the microbial community was determined by PLFA (Pawlett et al., 2013). Additionally, the fatty acid 18:2Ω6,9 was used as indicator of the incidence of ectomycorrhizal fungi, and the sum of the mol% of the fatty acids 15:0a, 15:0ai, 15:0, 16:0i, 16:1Ωc, cyc17:0 isomer, and 17:0ai as an indicator of total bacteria (Frostegard, 1996). The functional profile of the microbial community was determined by MSIR (Multiple Substrate Induced Respiration) using RABIT (Rapid Automated Bacterial Impedance Technique, Don Whitley, UK) (Pawlett et al., 2013). Substrates used were D-(+)-glucose (75mM), L- arginine (15mM), L-glutamine (15mM), citric acid (100mM), alpha-Ketoglutaric acid (100mM), and deionised water for basal respiration measurement. Aggregate stability was determined using a gravity-fed rainfall tower to simulate a natural rainfall event (Cooper, 2007). During the test, two rainfall intensities were used: 86.8 mm h⁻¹ and 47.7 mm h⁻¹, corresponding respectively to 1-50 years storm in and 1-10 years return storms for the River Lugg catchment. Statistical analysis was performed using the software STATISTICA 12 (Dell Inc. 2015). One-way ANOVA followed by post-hoc Fisher LSD comparison was performed to find homogenous groups. Significant difference was set at p<0.05. Principle Component Analysis (PCA) was used to analyse the multivariate PLFA and MSIR data. Resultant PCA factors scores were analysed for significant differences using ANOVA.

Results

There were no significant differences in microbial biomass between the treatments either in November 2015 or March 2016 ($p>0.05$), Table 1. In addition, there was no significant treatment effect on aggregate stability for either of the two rainfall intensities used ($p>0.05$), Table 1.

Table 1: Microbial biomass ($\mu\text{g C g}^{-1}$) and unstable aggregates (% weight g).

	Microbial biomass ($\mu\text{g C g}^{-1}$) $\pm$ SE		Unstable aggregates (% g) $\pm$ SE	
	November 2015	March 2016	1-50-yr storm	1-20-yr storm
Control, no CCs	226.1 $\pm$ 10.1	166.5 $\pm$ 9.7	50.2 $\pm$ 5.8	36.8 $\pm$ 10.5
<i>L. multiflorum</i>	232.3 $\pm$ 10.5	189.9 $\pm$ 10.8	47.9 $\pm$ 2.1	55.2 $\pm$ 5.7
<i>L. multiflorum</i> & <i>T. alexandrinum</i>	204.7 $\pm$ 15.3	171.2 $\pm$ 8.6	48.3 $\pm$ 3.8	41.3 $\pm$ 8.6
<i>L. multiflorum</i> & <i>V. villosa</i>	237.0 $\pm$ 10.9	191.9 $\pm$ 4.7	44.6 $\pm$ 1.6	27 $\pm$ 7.1

CCs cover crops, SE standard error

Cover crop choice affected the MSIR functional profile of the microbial community ($p<0.01$). In March 2016, the treatment *L. multiflorum* & *V. villosa* was significantly different from the other treatments on PC4, Figure 1a. This was mostly due to the basal respiration rate (loading >0.6). The first four principle components (PC) accounted for 86% of the total variation (PC1 31%, PC2 22%, PC3 19%, and PC4 15%). Cover crop choice also affected the phenotypic (PLFA) profile of the microbial community ($p<0.001$). In March 2016, the treatment *L. multiflorum* & *T. alexandrinum* had a significantly different profile on PC1 compared to the other treatments, Figure 1b. On PC1, fatty acids with loadings ≥ 0.8 included: 14:0, 15:0i, 15:0ai, 15:0, 16:0i, 16:1 ω 7c, and Me 17:0 isomer; 18:1 ω 9c had a loadings ≤ -0.8 . PC1 to PC3 accounted for 59% of the total variation (PC1 29%, PC2 19%, PC3 10%).

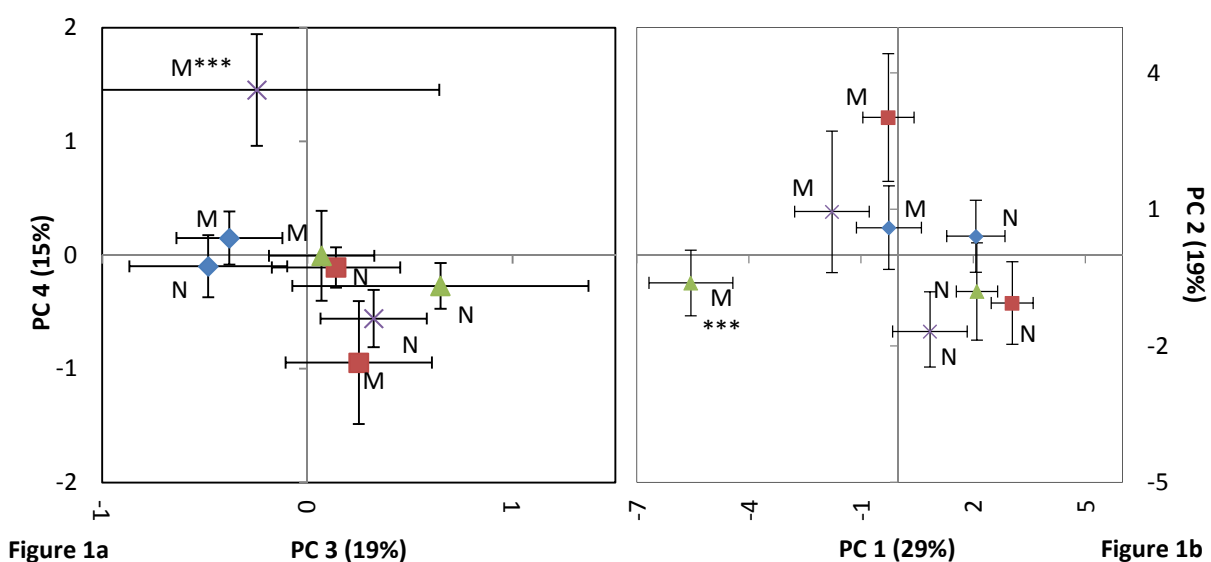


Figure 1: PCA score plots of the means ($n=5 \pm$ SE). Figure 1a: MSIR profile, PC 3 and 4. Figure 1b: PLFA profile PC 1 and 2. Diamonds are control, squares *L. multiflorum*, triangles *L. multiflorum* & *T. alexandrinum*, exes *L. multiflorum* & *V. villosa*. N=November 2015, M=March 2016. ***=significant differences ($p<0.05$).

In March 2016, relative quantities of the ectomycorrhizal fungi (indicated by the %mol of the fatty acid 18:2 ω 6, 9) were significantly greater (+40%) in the presence of CCs compared to the control, Table 2. Total bacteria (%mol) were significantly reduced (-21%) where *L. multiflorum* & *T. alexandrinum* were grown compared to other treatments. The fungi/bacteria ratio was 45% lower in the control compared to CCs treatments, Table 2.

Table 2: Fatty acids biomarkers (%mol) for fungi, total bacteria and fungi/bacteria ratio for the samples collected in March 2016.

	Fungi (18:2 ω 6,9)	Total bacteria	Fungi/bacteria ratio
Control, no CCs	2.5 $\pm$ 0.2 ^a	18.8 $\pm$ 2.1 ^a	0.13 $\pm$ 0.01 ^a
<i>L. multiflorum</i>	4 $\pm$ 0.3 ^{b,c}	19.1 $\pm$ 0.9 ^a	0.21 $\pm$ 0.02 ^b
<i>L. multiflorum</i> & <i>T. alexandrinum</i>	4 $\pm$ 0.2 ^b	16.2 $\pm$ 2.3 ^b	0.25 $\pm$ 0.02 ^b
<i>L. multiflorum</i> & <i>V. villosa</i>	4.6 $\pm$ 0.2 ^c	18.5 $\pm$ 2.6 ^a	0.25 $\pm$ 0.02 ^b

CCs cover crops, SE standard error, and letters represent homogenous groups

Conclusions

The absence of an effect of CCs on microbial biomass could be attributed to the short time (eight months) of the experiment. The majority of studies which reported an increase in microbial biomass were conducted over multiple growing seasons (Dinesh et al., 2006; Sainju et al., 2007). Therefore, if the experiment was conducted for longer, effects may become more pronounced. The lack of a significant effect on aggregate stability was attributed to the brief permanence of the CCs in the soil when the soil samples were taken (three months); other time-limited studies have come to similar conclusions (Welch et al., 2016). Studies reporting an increase of stable aggregates after CC implementation typically collect samples after longer periods, e.g. after CC termination (Hermawan and Bomke, 1997; García-González et al., 2016). The absence of differences in the first three PC of the MSIR profile could indicate that other soil properties, such as pH or total organic carbon, were the main drivers of the functional profile of the microbial community (Creamer et al., 2016), or that one season of CC implementation was too short to see an effect. However, the presence of a significant difference in PC4 for the treatment *L. multiflorum* & *V. villosa* in March 2016 could be an indication of an early-stage differentiation of the functional profile due to the CCs. It is also noticeable that the control treatment in November 2015 and the control in March 2016 were similar; while the functional profiles of the CCs in March 2016 appear to be diverging from November 2015, suggesting that a differentiation could be evident if the experiment was conducted for longer. The alteration of the relative abundance of the ectomycorrhizal fungi (18:2 ω 6, 9) and total bacteria in the presence of CCs in March 2016 demonstrate a microbial community shift towards fungal dominance. Similar conclusions were found in other studies (Nakamoto et al., 2012). However, this shift was captured by the PCA of the PLFA profiles only for *L. multiflorum* & *T. alexandrinum*, indicating an early stage of differentiation. The noticeable divergence of the CCs in the PCA score plot from November 2015 to March 2016 and the lack of separation between the controls support our hypothesis of a microbial community shift caused by the presence of CCs. Additional ongoing analysis of ergosterol and easily extractable glomalin may allow improved data interpretation. We concluded that the implementation CCs shifted the microbial community towards greater fungal dominance after one season, which in the long term could result in an increase of aggregate stability and therefore a decrease of soil erodibility and could improve sustainable maize cultivation. The lack of significant effects on microbial biomass and aggregate stability, and the small effects on the functional profile of the microbial community are to be attributed to the short observed time. This research suggests that it

may be necessary to implement conservation practices over a longer term in order to see changes in soil health.

Acknowledgments

I would like to thank the Douglas Bomford Trust and the Wye and Usk Foundation for sponsoring the project, and the British Society of Soil Science for funding my participation to this conference.

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Appendix C Crop production in Southern Britain conference paper

Field trial to investigate the role of cover crops in reducing runoff and soil erosion in maize

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Summary

This research investigated the role of different cover crops (CC) towards reducing runoff and soil erosion from maize. An eight-month field trial was undertaken whereby runoff was collected from bounded erosion plots. Treatments comprised (n=5): i) *Lolium multiflorum*, ii) *Lolium multiflorum* and *Vicia villosa*, iii) *Lolium multiflorum* and *Trifolium alexandrinum*, iv) control (no CC). The experiment was divided into three blocks according to in-field topography. Mean runoff was 1.8 times greater, and soil loss double, in control plots as compared to those with CC. This difference was not significant due to the high variability within each treatment caused by changes in plot micro-topography, which was evident from a different runoff flow direction between blocks. We concluded that the effect of CC on runoff and soil erosion on shallow slopes is not definitive, and further investigation is needed. Future assessments of soil erosion should take into account the variability of field micro-topography.

Key words: Maize, soil erosion, cover crops, micro-topography

Introduction

Surface water runoff and accelerated soil erosion often occur in maize cultivations in the UK, even on shallow slopes (Morgan, 2005). This poses a serious risk to water quality and riverine habitats, and often leads to degradation of soil health (Boardman, 2013). This erosion is mainly caused by the standard post-harvest management of leaving stubble over autumn and winter, which provides inadequate soil cover during the heavy rains associated with that period (Jokela *et al.*, 2009). Additionally, compaction caused by the late harvesting of maize often in wet soil conditions, facilitates runoff and thus erosion (Boardman & Poesen, 2006; Posthumus *et al.*, 2011). The cultivation of forage maize in the UK has increased from 29,000 ha in 2014 to 51,000 ha in 2016 (National Statistics, 2016), mainly for its use in anaerobic digestion (AD) for biogas production. Defra sees AD plants as pivotal to achieve the target 26% reduction of CO₂ UK emissions compared to 1990 levels by 2020 (Climate Change Act, 2008), thus encouraging future expansion of maize cultivation. However, if soil conservation measures are not implemented, the negative impacts of maize cultivation could outweigh future environmental benefits of biogas production. Cover crops, such as grasses, legumes, and cereals, are used as soil conservation practice for erosion protection and for soil nutrient management. Cover-cropping is used worldwide in maize cultivation, especially in the US (Brennan & Boyd, 2012; Dabney *et al.*, 2001; Snapp *et al.*, 2005), and it is also an emerging practice in the EU (Gabriel *et al.*, 2012; Gabriel & Quemada, 2011; Thorup-Kristensen *et al.*, 2003). The use of CC with maize in the UK could not

only decrease the offsite environmental costs of maize, but it could also be advantageous for UK farmers to enter agri-environmental schemes. For example Defra (2015) Good Agricultural and Environmental Conditions (GAEC) states: GAEC-4 - maintenance of a minimum soil cover, GAEC-5 - implementation of erosion control measures, and GAEC-6 - maintenance of soil organic matter. The selection of CC species needs to be fit to the management goals (e.g. soil erosion protection), soil type and the climatic conditions. Species choice is important as additional benefits other than erosion control can be achieved. For example, legumes, such as vetches (*Vicia sativa*, *Vicia villosa*) and clovers (*Trifolium repens*, *Trifolium pratense* and *Trifolium alexandrinum*) are used to restore soil fertility by fixing nitrogen (N) (Brennan & Boyd, 2012). Grasses, such as Italian ryegrass (*Lolium multiflorum*) and cereal rye (*Secale cereale*) are often selected for erosion control as they rapidly establish, protecting the soil from the direct impact of raindrops, have a fibrous root system that contributes to decreased soil erodibility, and have a high stem density which reduce runoff velocity (Liedgens *et al.*, 2004; Burney & Edwards, 2005). Other crops that contribute to erosion control are tap-rooted crops (e.g. Forage radish, *Raphanus sativus* and Rapeseed, *Brassica napus*), which increase water infiltration and decrease soil compaction (Chen & Weil, 2011; Pratt *et al.*, 2014), thereby reducing runoff. Oats (*Avena sativa*), barley (*Hordeum vulgare*) and winter rape (*Brassica rapa*), are few examples of CC also known as catch crops. They are used to decrease the leaching of nutrients such as phosphorous (P) and N over-winter (Meisinger *et al.*, 1991; Salmerón *et al.*, 2010 Gabriel *et al.*, 2012; Restovich *et al.*, 2012). Although the use of CC in the UK is still largely experimental, this practice is starting to gain prominence. Cover crops are promoted among maize growers by Catchment Sensitive Farming advisors and independent agronomists. More knowledge is required to demonstrate the potential of CC for reducing soil erosion while improving physical, chemical and biological aspects of soil quality in maize systems, on CC sowing and termination techniques and on CC resistance to herbicides. This research aimed to quantify the role of Italian ryegrass and mixtures of Italian ryegrass with legumes as compared with un-amended controls with regards to reducing surface runoff and soil loss in maize cultivation. It was hypothesised that the presence of CC would reduce runoff and soil loss as compared to control plots without CC, and that Italian ryegrass would decrease runoff and soil loss more than mixtures of legume-grass because it would provide a faster soil cover due to its rapid growth. Field dominant slope and the runoff flow direction were also assessed to determine whether any underlying micro-topographical variation was affecting experimental variables.

Materials and Methods

The experiment ran from July 2015 to March 2016 within a field of commercially grown maize (Herefordshire, UK: OS grid references: 344505 E, 257646 N) (Fig. 1). Annual average rainfall was 793.8 mm yr⁻¹ (Met Office, 2016), soil was a sandy loam and the slopes ranged from 3° to 4°. The experiment, arranged in a randomised design, comprised four treatments replicated five times. Experimental treatments included: i) Italian ryegrass (*Lolium multiflorum*), ii) Italian ryegrass and hairy vetch (*Vicia villosa*), iii) Italian ryegrass and berseem clover (*Trifolium alexandrinum*), and iv) control (post-harvest wintered maize stubbles). The experiment was divided in three blocks according to site topography. These blocks are referred to as A (from plot 1 to plot 8), B (from plot 9 to plot 14) and C (from plot 15 to plot 20) (Fig. 1). The intention was to assess whether topographical variability at field-level was influencing experimental findings. Forage maize (Sergio & Kentaurus varieties) was drilled with a 75 cm row space in May 2015. Cover crop options were broadcast by hand at the end of June in strips inside the erosion plots (12 m × 1.5 m), which were oriented parallel to the maize rows and to the direction of the dominant slope. Seeding rates were double the standard seeding rates for the single species to insure crop establishment: i) Italian ryegrass 56 kg ha⁻¹, ii) Italian ryegrass and hairy vetch 56 kg ha⁻¹ and 62 kg ha⁻¹, and iii) Italian ryegrass and Berseem clover 56 kg ha⁻¹ and 59 kg ha⁻¹. The dominant slope across the plot areas was not significantly different between treatments or between blocks.

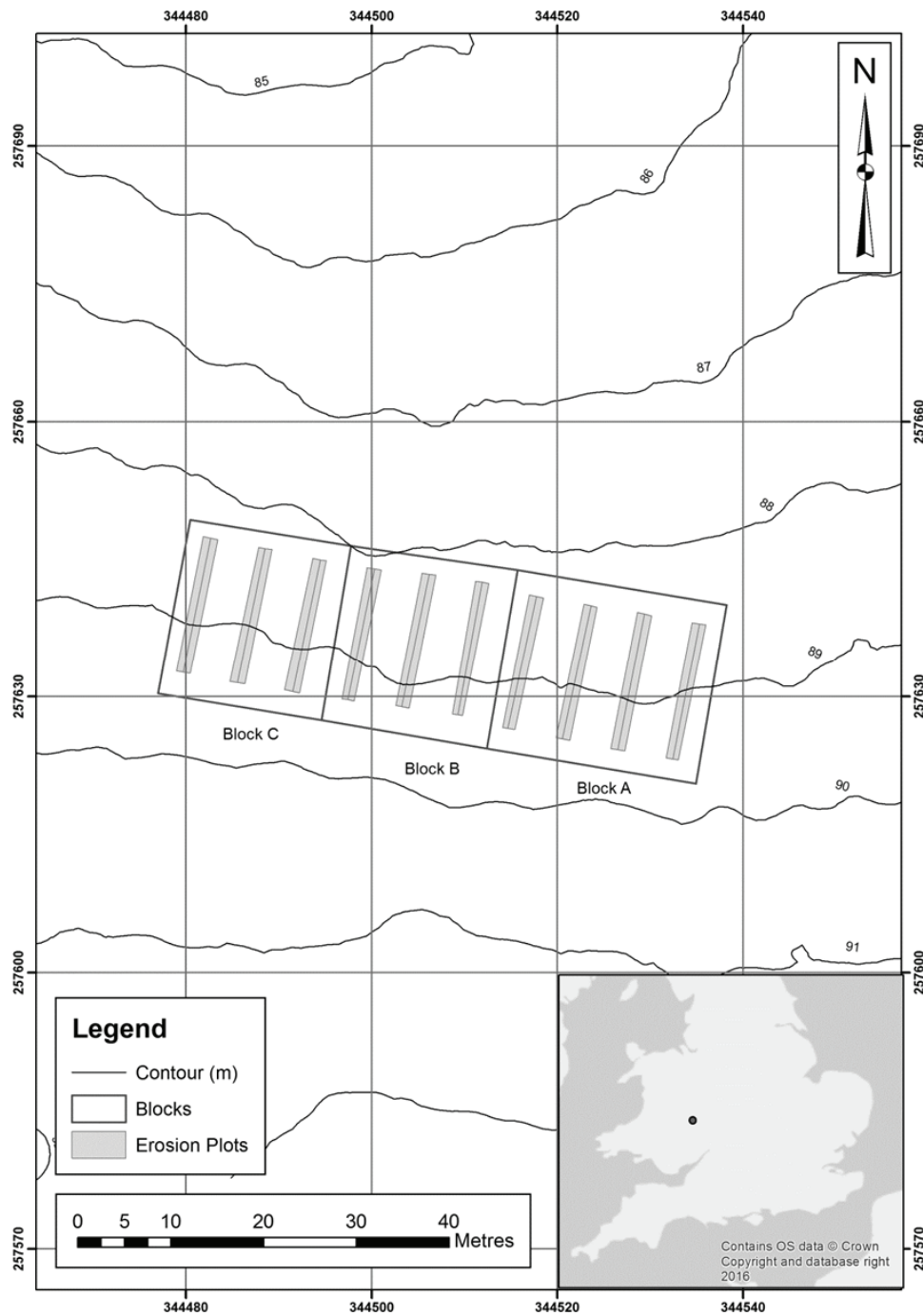


Fig. 1. Map of the field trial showing the three zones identified to assess the effects of topography.

Runoff and sediment sampling

Plots were hydrologically isolated from field runoff by soffit boards dug into the soil (to a depth of *C.* 0.2 m) on three sides. Runoff and sediments were collected by a stainless steel Gerlach trough placed at the bottom of the plots connected with a 68 mm diameter pipe to a water tank (220 L capacity). Tanks were sampled five times during the 8-month experimental period (Table 1). At each sampling event, water level was recorded and after agitation, runoff and sediment samples were collected using three 500 mL sub-sample bottles, kept at 10 cm distance from the bottom of the tank. Where runoff level in the tank was very low, runoff was collected with a scoop. Following sampling, the tanks were emptied in preparation for the next sampling period. Subsamples (250 mL) of the runoff collected for each tank were oven-dried (105°C for 12 h) to measure the total sediment load (Clesceri *et al.*, 1999). The amount of sediment present in the runoff was use to estimate soil loss per hectare.

Table 1. *Runoff collection periods*

Runoff collection period	Sampling event
28 July 2015 – 15 October 2015	1
27 November 2015 – 21 December 2015	2
21 December 2015 – 13 January 2016	3
13 January 2016 – 16 February 2016	4
16 February 2016 – 15 March 2016	5

Flow direction analysis

Elevation point measurements were taken every 7–10 m across the field before the implementation of the field trial with a Trimble GeoXH, which is equipped with real time kinematic corrections and a horizontal and vertical precision of <3 cm. After the establishment of the erosion plots, erosion plot corners and their elevation were also recorded as described before. Analysis of the topographic properties of the field was carried out using ArcGIS software (ESRI), version 10.2. A surface elevation raster with a resolution of 3.0 m was extrapolated from the survey points using an Inverse Distance Weighted interpolation technique. A resolution of 3.0 m was deemed suitable for the surface raster based on the original point measurement resolution and to maintain micro-topographic features of interest within the field. The flow direction tool within ArcGIS was applied to create a raster representing the direction of the runoff flow. The tool outputs one of eight cardinal directions (N, NE, E, SE, S, SW, W, and NW) for each cell in the raster, denoting the direction of surface flow from that grid cell to one of its immediate neighbours. The flow direction raster was converted to a polygon feature, which was overlain and intersected by the demarcated plot boundaries. Subsequently, the area (%) of each flow direction class (N, NE, E, SE, S, SW, W, and NW) within the plots was calculated. Total area extent of each flow direction class was compared between treatments and between the blocks.

Statistical analysis

Statistical analysis was performed using the software STATISTICA 12 (Dell Inc, 2015). One-way ANOVA follow by post-hoc Fisher LSD analysis was performed on the runoff and soil loss data to find homogenous groups. The level of significance was set at 0.05. Pearson product - moment correlation coefficient (r) was used to determine linear correlations between runoff and soil loss. Since the two tests assume normality and homogeneity data was normalised using Box-Cox transformation (Mancini *et al.*, 2017).

Results

Experimental manipulations did not affect runoff flow direction but there was a significant difference ($P<0.05$) in flow direction between blocks. The area of the flow direction class NW in block C was significantly smaller than in block A. The area of the flow direction class N in block C was significantly smaller than in block A and B. The area of the flow direction class NE in block C was significantly greater than in block A and B (Mancini *et al.*, 2017). These results confirmed the presence of a depression in the area occupied by block B, which was previously noticed by in-field observations. There were no significant differences in runoff and soil loss between the treatments during any collection period (Mancini *et al.*, 2017). In addition, there were no significant treatment effects for either total cumulative runoff or soil loss throughout the duration of the experiment (Table 2). Significant differences were found between the treatment blocks for total cumulative runoff and soil loss (Table 3). Cumulative runoff collected in block C ($34 \text{ m}^3 \text{ ha}^{-1} \pm 28$) was significantly lower

compared to the runoff collected in block B ($187 \text{ m}^3 \text{ ha}^{-1} \pm 32$). Cumulative soil loss in block C ($48 \text{ kg ha}^{-1} \pm 44$) was significantly lower than cumulative soil loss in B ($233 \text{ kg ha}^{-1} \pm 67$). Runoff and soil loss for each sampling period, their cumulative values and the runoff flow direction classes were compared using Pearson product - moment correlation coefficient (r). Runoff and soil loss for each sampling period were linearly correlated $r > 0.9$ ($P < 0.05$: Table 4).

Table 2. *Cumulative runoff and soil loss (mean $\pm$ standard error) comparing experimental treatments*

	Cumulative Runoff ($\text{m}^3 \text{ ha}^{-1}$) $\pm$ SE	Cumulative soil loss (kg ha^{-1}) $\pm$ SE
Control	149 ± 61^a	210 ± 98^a
<i>L. multiflorum</i>	99 ± 39^a	90 ± 38^a
<i>L. multiflorum</i> & <i>T. alexandrinum</i>	103 ± 58^a	183 ± 108^a
<i>L. multiflorum</i> & <i>V. villosa</i>	46 ± 24^a	39 ± 22^a
CC treatments	83 ± 24^a	104 ± 39^a

CC cover crops, letters represent statistical groupings.

Table 3. *Cumulative runoff and soil loss (mean $\pm$ standard error) comparing blocks*

	Cumulative Runoff ($\text{m}^3 \text{ ha}^{-1}$) $\pm$ SE	Cumulative soil loss (kg ha^{-1}) $\pm$ SE
Block A	$82 \pm 38^{a, b}$	$116 \pm 68^{a, b}$
Block B	187 ± 32^a	233 ± 67^a
Block C	34 ± 26^b	48 ± 44^b

Letters represent statistical groupings.

Table 4. *Pearson product - moment correlation comparing soil loss and runoff.*
Soil loss 1 = soil loss during sampling period 1

y	x	Equation	r
Cumulative soil loss	Cumulative Runoff	$y = 0.97 \times -47$	0.98
Soil loss 1	Runoff 1	$y = 0.43 \times -0.03$	0.94
Soil loss 2	Runoff 2	$y = 0.9 \times -0.2$	0.96
Soil loss 3	Runoff 3	$y = 1 \times -0.31$	0.96
Soil loss 4	Runoff 4	$y = 1.04 \times -0.04$	0.98
Soil loss 5	Runoff 5	$y = 0.98 \times -0.03$	0.96

Discussion

Contrary to the hypotheses, although mean runoff and soil loss were 1.8 and 2.0 times greater in the control plots as compared to the CC treatments, none of the CC treatments statistically affected either runoff production or soil loss. The lack of significant differences between treatments was likely due to high variability (SE) within the treatments caused by field spatial heterogeneity. Differences in field micro-topography led to a differentiation of the runoff flow direction, which became the main factor that contributed to the variation between the plots. Runoff and soil loss collected in block C were significantly lower than in block B. This was in large part due to the micro-topography of the

field, which probably facilitated the convergence of sub-surface soil moisture in the depression that was block B, decreasing soil infiltration in that area and therefore increasing surface runoff and soil erosion. Additionally, the presence of subsidiary slopes inside the plots in block A and C led to an underestimation of the runoff and soil loss collected in those blocks, adding to the variation within the treatments. Contributing factors to the lack of differences between Italian ryegrass and the mixtures of Italian ryegrass with legumes were probably the dominance of the grass in the grass-legumes plots, which was observed in the spring, and the double total seed rate of the grass-legumes plots compared to the grass-alone plots. In light of these results, competition between species needs to be taken into account in the future when choosing CC mixtures and further investigation on CC sowing rates is needed. Field-based measurements of erosion and runoff have always been challenging due to: i) the extreme complexity and the scale-dependency of the erosion process, ii) the natural variability of the soil environment and iii) inevitable disturbance caused by the design of the erosion plots and by field operation, e.g. harvest (Boix-Fayos *et al.*, 2006; Gómez *et al.*, 2005). Consequently, many papers have recorded high variability in runoff and soil loss within the same treatment, despite a statistically appropriate number of replicates (up to 40 in Gómez *et al.*, 2001), refer to Rüttimann *et al.* (1995) and Hjermfelt & Burwell (1984) for other examples. This paper did not aim to review the advantages and disadvantages of using a certain type and size of erosion plots (refer to Boix-Fayos *et al.* (2006) and Kinnell (2016)) or to argue if they are adequate to measure runoff and soil erosion (refer to Wainwright *et al.* (2000) and Boix-Fayos *et al.* (2006)). However, future measurements of in-field soil erosion should undertake detailed assessments of within plot micro-topography as support for explaining experimental results. Many studies, have reported that CC caused a significant decrease of soil loss and runoff, see Dabney *et al.* (2010) and Delgado *et al.* (2007); nevertheless CC effects in this project were obscured by the micro-topography of the field, further investigation is therefore needed to understand the potential of CC use in the UK in maize systems.

Acknowledgements

The authors would like to acknowledge the Wye and Usk Foundation and the Douglas Bomford Trust for the financial and moral support for the project. Thank you also to the farmer and landowner who kindly allowed us to use the field and to all the farm workers who help us during the experiment. A special thank you goes to all Cranfield University research students and staff members, who supported us in the realisation of the experiment.

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Appendix D Python script


```
# -*- coding: utf-8 -*-
```

```
"""
```

Created on Thu Jan 28 15:39:22 2016

```
@author: ns08dms
```

```
"""
```

```
import cv2
```

```
import numpy as np
```

```
from scipy.spatial import distance
```

```
from skimage import transform as tf
```

```
from skimage.draw import circle_perimeter
```

```
def warp_img(corners, img):
```

```
    # Order corners
```

```
    dst = sortpts_clockwise(np.array(corners).reshape(4, 2))
```

```
    # Output size
```

```
    w = dst[:, 0].max() - dst[:, 0].min()
```

```
    # Transform
```

```
    src = np.array([0, 0, 1, 0, 1, 1, 0, 1]).reshape(4,2) * w
```

```

tform = tf.ProjectiveTransform()

tform.estimate(src, dst)

warped = tf.warp(img, tform, output_shape = (w, w))

return warped


def get_corners2(img, fn = None):

    # Try using contours

    r = img[:, :, 2]

    g = img[:, :, 1]

    b = img[:, :, 0]


    # First threshold the red (being generous)

    thresh = np.array((r > 200) & (g < 200) & (b < 200))

    r = thresh * r

    g = thresh * g

    b = thresh * b


    r[r > 0] = 255


    # find contours in the edge map

```



```
(cnts, _) = cv2.findContours(r.copy(), cv2.RETR_EXTERNAL,  
cv2.CHAIN_APPROX_SIMPLE)
```

```
centres = []
```

```
if fn:
```

```
    out = np.zeros_like(img)
```

```
for c in cnts:
```

```
    # approximate the contour
```

```
    peri = cv2.arcLength(c, True)
```

```
    approx = cv2.approxPolyDP(c, 0.01 * peri, True)
```

```
    # Some filtering rules
```

```
    # 1. The targets are square
```

```
    # 2. They are solid
```

```
    # 3. They have a minimum size
```

```
    (x, y, w, h) = cv2.boundingRect(approx)
```

```
    aspectRatio = w / float(h)
```

```
    area = cv2.contourArea(c)
```

```
    hullArea = cv2.contourArea(cv2.convexHull(c))
```

```
if hullArea == .0:
```

```
    solidity = .0
```

```
else:
```

```
    solidity = area / float(hullArea)
```

```
# Criteria
```

```
keepDims = w > 100 and h > 100
```

```
keepSolidity = solidity > 0.9
```

```
keepAspectRatio = aspectRatio >= 0.7 and aspectRatio <= 1.3
```

```
if keepDims and keepSolidity and keepAspectRatio:
```

```
    #cv2.drawContours(out, [approx], -1, (255, 255, 255), -1)
```

```
    M = cv2.moments(approx)
```

```
    (cX, cY) = (int(M["m10"] / M["m00"]), int(M["m01"] / M["m00"]))
```

```
    centres.append((cX, cY))
```

```
if fn:
```

```
    cv2.drawContours(out, [approx], -1, (255, 255, 255), -1)
```

```

if fn:

    cv2.imwrite(fn, out)

if len(centres) < 4:

    return None

return centres

def sortpts_clockwise(A):

    #      http://stackoverflow.com/questions/30088697/4-1-2-numpy-array-sort-
    clockwise

    # Sort A based on Y\(col-2\) coordinates

    sortedAc2 = A\[np.argsort\(A\[:,1\]\),:\]

    # Get top two and bottom two points

    top2 = sortedAc2\[0:2,:\]

    bottom2 = sortedAc2\[2:,:\]

    # Sort top2 points to have the first row as the top-left one

    sortedtop2c1 = top2\[np.argsort\(top2\[:,0\]\),:\]

    top\_left = sortedtop2c1\[0,:\]

```

```

# Use top left point as pivot & calculate sq-euclidean dist against
# bottom2 points & thus get bottom-right, bottom-left sequentially
sqdists = distance.cdist(top_left[None], bottom2, 'sqeuclidean')
rest2 = bottom2[np.argsort(np.max(sqdists,0))[:-1],:]

# Concatenate all these points for the final output
return np.concatenate((sortedtop2c1,rest2),axis =0)

def draw_grid(image, origin, nrows, ncols, xdist, ydist, circ_size):
    oy, ox = origin

    copy = image.copy()

    for i in xrange(1, nrows):

        y = oy + i * ydist

        for j in xrange(1, ncols):

            x = ox + j * xdist

            rr, cc = circle_perimeter(y, x, circ_size)

            copy[rr, cc, :2] = 0

            copy[rr, cc, 2] = 255

```

```
rr, cc = circle_perimeter(y, x, circ_size + 1)

copy[rr, cc, :2] = 0

copy[rr, cc, 2] = 255


return copy
```

