博士論文

不耕起早生栽培における雑草マルチを使用した持続可能な窒素管理

Sustainable nitrogen management using weed mulch in no-tillage conservation agriculture system

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Contents

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Summary

Agriculture is a significant prerequisite for sustaining human life on earth. The increasing human population is exerting pressure on limited agriculture lands to produce more food resources, which has led to the development and usage of agriculture inputs such as chemical fertilizer, pesticides, and herbicides. The application of these synthetic products on agriculture lands increased the yield and was able to sustain the growing human population but was met with some consequences. The extensive use of chemical fertilizers degraded the environment causing problems such as soil degradation and water pollution. Besides the environmental impacts, human health hazards and economic loss are also some of the major concerns. Unregulated and excessive application of fertilizer in modern agriculture practice is unsustainable, and the resources are not being efficiently utilized to produce agriculture products. Hence, a sustainable agriculture approach with efficient resource utilization is required for long-term agricultural sustainability.

Conservation agriculture (CA) is one of the sustainable agriculture practices, which consists of three principles; 1) Minimum tillage; reduces the intensive and aggressive use of land, 2) crop cover and mulching; conserve the soil from organic matter, and 3) crop rotation; increases soil fertility. CA is a soil-based agriculture system that conserves the soil ecosystem and encourages better nutrient cycling. This studies on this thesis is based on no-tillage with weed management (NTW) system, which follows the principles of CA. The distinct characteristic of NTW is the naturally occurring weeds, which are grown together with the crops. Generally, weeds are considered a detrimental factor in the agriculture system because weeds compete with crops for water, nutrient, and sunlight. Weeds are usually controlled by using herbicides, which harms the crops and as well as to the surrounding environment. However, NTW controls and utilizes weeds as a nitrogen resource by slashing and mulching practice. Therefore, using the weeds that are available within the field as a nitrogen resource

may minimize the need for external input and cost, eventually reducing the N loss from the agriculture lands.

This thesis consists of three field experiments on the NTW system. In the first experiment in Chapter 2, short-term impact of tillage on commercial NTW farm and its soil ecosystem is investigated. Here, the severity and immediate effects of tillage on the NTW soil ecosystem is demonstrated. The research site was managed by NTW practice for 30 years, and the impact of tillage on soil ecosystem was immediately observed within one year. A substantial amount of time and soil management practice is known to require for conserving the soil and transition to no-tillage system, but the disruption through tillage was an instantaneous process. Reduction in soil organic matter, soil macrofauna, and a significant decrease in earthworm biomass were observed after the introduction of tillage. In this chapter, the short-term impact of tillage on NTW farm was studied where the soil organic matter and soil animals significantly reduced after tillage practice.

In Chapter 3, nitrogen release from weeds to the soil in NTW system was investigated by introducing three frequencies (treatments) of weed slash and mulch practice. Three treatments were established where weeds were slashed zero (S0), once (S1), and twice (S2) with soybean plantation. The slashed weeds were used as mulch resources in the respective treatments. The N release from slashed weed was calculated by conducting a litterbag experiment. The decomposition and N release of a model plant (*Imperata cylindrica*) was measured, and N released from slashing weeds was estimated. Contrary to the senescent grass litter, the green *I. cylindrica* litter released N to the soil for the first two months of decomposition. Treatments S1 and S2 yielded the same amount of soybean compared to S0 treatment. The result suggests that the slashing weed can be reduced to one time, which reduces the labor for weed management. The amount of weed used as mulch increased with the increasing frequency of slashing, which increased soil microbial biomass and N release from the weeds to the soil. Here, the results demonstrated that the weeds can be controlled by slashing and were used as a mulching resource, which released N back to the soil.

In Chapter 4, the nitrogen budget in NTW systems was measured and compared with tillage treatment. The nitrogen fertilizer (50 kg/ha) was added in both tillage and NTW treatments. Control treatments were also established where no fertilizer was used. The weeds in no-tillage plots were slashed manually and were mulched on the soil surface. The results showed a similar amount of wheat production in both tillage and NTW treatments. However, control plots showed significantly less wheat production, which suggests fertilization is necessary. The nitrogen use efficiency (NUE) for the winter wheat crop was higher in NTW treatment which means that applied fertilizer was efficiently used in NTW compared to the tillage treatment. The total soil nitrogen concentration also increased in no-tillage treatments, which increases the available nitrogen in the soil. Higher root biomass and soil animal activity were also observed in NTW treatment that may have resulted in high soil N. The results showed that even through NTW system consisted of weeds, crops were able to uptake more nitrogen and efficiently utilize N compared to the crops in conventional tillage practice.

The efficient management of N in agriculture land with locally available resources can reduce the N loss that can conserve the environment and simultaneously improve the livelihood of producers due to less dependency on external inputs. The weeds that were available freely within the field were used as an alternative source of N input in the agriculture land. Weeds are generally considered as a detrimental factor in agriculture due to its competitive nature with crops for water, nutrients, and other resources. Nevertheless, the results show that weeds can be utilized to create an N efficient agriculture system. Weeds can take up the available nitrogen from the soil and can act as an N sink reducing the N loss from the soil. These weeds are later slashed and mulched, which releases N back to the soil. Even though the amount of N release from weeds was low, weeds recycled and contributed some

N in the agriculture field, reducing the loss to the environment. This can potentially be beneficial for resource-poor small-scale farmers and reduce their input costs. Further researches in the NTW system, such as a combination of reduced fertilizer or compost with weed mulch, can be considered as an alternative to increasing the crop yields as well as the NUE.

Chapter 1

General Introduction

The population of the world is expected to reach 9.1 billion by 2050 (FAO, 2009), and there are increasing demands on agricultural systems to produce greater yields through the use of limited natural resources (Godfray et al., 2012). Hence, efficient resource management of agriculture amendments is an important task to reduce the negative impact on the environment. Sustainable management of the resource in agriculture can aid resource-poor small-scale farmers to be less dependent on anthropogenic inputs improving agriculture conditions in local as well as global scale.

Sustainable agriculture practices such as organic farming and conservation agriculture are being practiced around the globe. However, these practices still face challenges such as nutrient loss through tillage in organic agriculture and weed control and resource management in conservation agriculture (CA). No-tillage with weed (NTW) management system, which is a category of conservation agriculture that utilizes weeds to manage the nutrient cycle in the agriculture system. Weeds increase plant diversity in the agriculture field and may be able to reduce nutrient loss through resource acquisition, which may not be accessible to crops. Weeds compete with crops for sunlight, water, nutrient, and other resources. Therefore, the weeds are slashed and mulch in the soil that can release the nutrient back to the soil. Hence, NTW might be an alternative agriculture practice that can promote sustainable resource utilization in the agriculture field.

In this chapter, researches on resource utilization practices of sustainable agriculture and agriculture, in general, are reviewed. Then, the hypothesis that is investigated in the studies is stated.

Nitrogen for the plant's growth and agriculture green revolution

Nitrogen (N) is an essential element for plant growth, as a building block of amino acids and proteins. Nitrogen is the most abundant element in the atmosphere but is in an inactive state and is unusable by plants directly. Hence, nitrogen needs to get transformed or fixed into a reactive state. Reactive nitrogen (Nr) is biologically, chemically, and radiatively active nitrogen compounds in the atmosphere and biosphere. Nr includes a wide range of nitrogen forms such as ammonium (NH_4^+), nitrate (NO_3^-), nitrogen dioxide (NO_2), and organic compounds like urea and amines (United Nations Environment Programme and Woods Hole Research Center, 2007). In nature, nitrogen is fixed by leguminous plants, micro-organisms in the soil, and by lightning (Canfield et al., 2010). However, naturally formed reactive nitrogen is not sufficient to produce enough food to support the increasing global human population. Production of reactive N fertilizers by the Haber–Bosch process was, therefore, one of the most important inventions of the 20th century, thus resulting in a stable increase in the agriculture food production to support the world population (Smil, 1999). The influence of artificially produced N fertilizer on agriculture production was prominent during the green revolution (GR) (Tilman, 1998). The GR initiatives increased agriculture production worldwide through the adoption of the first generation of agrochemical inputs (fertilizer and pesticides) and high yield variety crops for 25 years from 1965 to 1990 (Murgai et al., 2001; Spielman and Pandya-Lorch, 2009; Hazell, 2010). So, GR was able to achieve its goal of food production and ensure global long-term food security.

Intensive use of land and nitrogen management in the conventional agriculture system

The doubling of agricultural food production worldwide over the past four decades has been associated with a 7-fold increase in the use of nitrogen fertilizers (Galloway et al., 2008). Consequently, both the recent and future intensification of the use of N fertilizers in agriculture already has and will continue to have major detrimental impacts on the diversity and functioning of soil micro-organisms, animal, and plant ecosystems (Gruber and Galloway, 2008; Mazzoncini et al., 2011). The availability of inexpensive nitrogen fertilizer all over the world made farmers apply excessive amounts of nitrogen in their farmland (Cassman et al., 2002). Along with the practices such as intensive tillage, using heavy machinery has led to the loss of nitrogen from the soil ecosystem due to soil denitrification, erosion, runoff, leaching, and volatilization (Raun and Johnson, 1999). In recent years, a large gap has been observed between the amount of nitrogen fertilizer added to the agricultural land and the amount of nitrogen used by the crops (Tilman et al., 2002). Studies have calculated that about 50-70 % of applied N fertilizer is lost to the environment (Bradley and Kindred, 2009; Hodge et al., 2000; Raun and Johnson, 1999). Economically, the N loss represents a $15.9 billion annual loss of N fertilizer and that even a 1% increase of nitrogen use efficiency (NUE) would result in global savings of $234 million (Raun and Johnson, 1999). Furthermore, the typical impact of excessive use of N fertilizer on the environment consists of eutrophication of freshwater and marine ecosystems (Beman et al., 2005). Hence, researchers suggest that the best hope for reducing the need for fertilizer N lies in finding more efficient ways to deliver fertilizer to the crops. It is, therefore, of major importance to identify the critical steps of controlling plant NUE. Moll *et al.* (1982) defined NUE as being the yield of grain per unit of available N in the soil (including the residual N present in the soil and the fertilizer). The objective of evaluating nutrient use efficiency is to increase the overall performance of cropping systems by providing economically optimum nourishment to the crop while minimizing nutrient losses from the field and supporting agricultural system sustainability through contributions to soil fertility or other soil quality components. In order to solve the agriculture-derived nitrogen problem, it is necessary to improve the nitrogen utilization rate of soil and crops in agricultural land and to reduce nitrogen fertilizer usage (Canfield et al., 2010; Tilman et al., 2002).

Nitrogen use efficiency is extensively studied topic in conventional agriculture systems, and there are many studies and methods to optimize NUE depending on the improved crop cultivar, land management, and fertilizer input methods. Two decades ago, the NUE of the world for cereal crops was 33% (Raun and Johnson, 1999), which means that 67% of the applied fertilizer was lost to the environment. Modern practices such as prescriptive fertilizer management, using enhanced fertilizer, GMOs for increasing biomass and grain yield, nitrification inhibitors are used to increase the NUE (Garnett et al., 2015; Giller et al., 2004; Liu et al., 2015; Sharma and Bali, 2018; Shoji et al., 2013; Vitousek et al., 1997). Ecological methods like legume plantation, cover crops, no-tillage, conservation agriculture also have been studied as alternative way to increase NUE (Choi et al., 2016; Qin et al., 2015; Tai-wen et al., 2018). A recent global study on cereal NUE showed that by 2015, the world NUE was improved by only 2% (35.2%) (Omara et al., 2019). In most cases, improvement in NUE requires the investment of resources on external factors like enhanced crop cultivars and fertilizer or usage of cover crops/mulch, herbicides, and pesticides. However, these practices may not be suitable for the long-term sustainability of the soil ecosystem because of the degradation of soil due to the intensive use of land and external inputs. Furthermore, dependency on the external resource means that these are applicable only in selected areas and might not be efficient for small scale farmers in resource-poor areas. Hence, an alternative practice that can be applied both on a global and local scale is required to increase NUE worldwide.

Conservation agriculture

Conservation agriculture (CA) was developed to establish a sustainable and economical alternative to modern conventional farming practices. Conservation agriculture is most suitable for smallholder farmers representing a combination of agronomic technologies, which include minimum disturbance of soil, conservation of soil by mulching with residues, and diversification of cropping system. While modern agricultural systems have resulted in soil degradation due to intensive use of land and chemical inputs, the adoption of CA technology has improved conditions in such systems (Derpsch & Friedrich, 2009; Hobbs et al., 2008). Worldwide, there are 500 million smallholder farms, in which 80 percent of the food produced is consumed within Asia and Africa (IFAD, 2011). In CA, the land is prepared with minimal soil disturbance, which is essential to maintaining minerals within the soil, stopping erosion, and preventing nutrient loss from occurring within the soil. The second principle consists of permanent soil cover by organic matter that conserves the soil and reduces nutrient loss through natural factors such as rain, wind, and sun. It also provides nutrition to the micro and macro-organisms in the soil and optimizes the microclimate in the soil for optimal growth and development of soil organisms, including plant roots. The third principle is the practice of crop rotation with more than two species. The rotation of crops will not allow pests and pathogens such as insects and some weeds to be attached to specific crops (Hobbs et al., 2008). Also, different plants have different root depths and nutrient requirements, which are capable of exploring different soil layers for resources. Adapting these practices was found to improve soil fertility, and crop performance (Pullaro et al., 2006), and the most important principle of the CA is that it can be applicable to all agricultural landscapes and can be adapted with local practices (FAO, 2008). CA has direct impacts on the livelihood of the farmers because of the reduced labor requirements for tillage, land preparation, and reduces cost for managing the farm. Hence, CA is a promising agriculture practice for efficient nutrient management based on its three principles.

Resource management and NUE in conservation agriculture

Long term (5-10 years) adoption of conservation agriculture practice can measurably enhance the quantity and quality of soil organic matter (Amado et al., 2006; Bai et al., 2009; He et al., 2011; Hobbs, 2007; Kay and VandenBygaart, 2002). The impact of soil change on plant NUE and N fertilizer management in the CA system is a complex process, and studies have shown contrasting results. Many of these studies focus on different tillage practice combined with crop rotations (Al-Kaisi and Kwaw-Mensah, 2007; Halvorson et al., 2004; López-Bellido and López-Bellido, 2001) or in combination with residue management and cover crops (Angás et al., 2006; Burgess et al., 2002; Choi et al., 2016; Qin et al., 2015). Some conservation agriculture practice like raised bed approach has found higher NUE and two times lower N loss compared to conventional plow tillage (Devkota et al., 2013). The conflicting results may be due to the efficiency of the fertilizer and application time in the cropping seasons or site-specific. The combination of fertilizer and residue retention has shown to reduce NUE since residues can increase temporary immobilization of fertilizer, which is released in the following years (López-Bellido and López-Bellido, 2001). The researches reviewed here are using different amounts of fertilizer and higher fertilizer, which can increase the N loss and reduce yield. Further improvement inefficient use of nitrogen in CA systems, N fertilizer application rate, type of fertilizer, the timing of fertilizer application and the method of fertilizer application and use of different cultivars are some of the discussed options (Table 1).

Table1. Different approaches to evaluate the efficiency of nitrogen in the CA system

Practice used to evaluate the efficiency of N input	**References**
Fertilizer placement in conservation agriculture	(Rao and Dao, 1996)
No-tillage, crop rotation with different rate of fertilization	(López-Bellido and López-Bellido, 2001)
	(Torbert et al., 2001)
High input fertilizer with conservation tillage	(Rusinamhodzi et al., 2011)
Permanent raised bed tillage with different rate of fertilizer	(Devkota et al., 2013)
Conservation tillage and residue management	(Brennan et al., 2014)
Minimum tillage with different cover crops	(Radicetti et al., 2016)
Use of external fertilizer and cover crops	(Sainju and Singh, 2008)
	(Habbib et al., 2016)

The cost of production is reduced mostly due to less dependency on fuel, labor, and water resource. CA resulted in improved nitrogen use efficiency (10-15%), which was mainly a result of the better placement of fertilizer with the seed drill as opposed to the traditional system. In some reports, nitrogen fertilizer efficiency was recorded as lower, a result of microorganisms tying up the nitrogen in the residue. However, in other longer-term experiments, the release of nutrients increased with time because of more active microbial activity and nutrient recycling (Carpenter-Boggs et al., 2003).

Plant diversity in the agriculture system

High plant diversity in the grassland ecosystem has shown to have high productivity, that further exhibits numerous ecological functions such as attracting highly diverse insects that feed on the plants that later attract predators creating a complex food chain in the system. The lack of human intervention conserves the soil and overall grass ecosystem from mechanical and other anthropogenic disturbances. The grassland ecosystem is a self-sustaining and nutrient efficient ecosystem with high productivity due to high plant diversity, complex food web structure, and better nutrient cycling (Craven et al., 2016; Glover et al., 2010; Tilman et al., 2001). On the other hand, conventional agriculture was developed to sustain human population and is a one-directional system. Other than edible plants, rest are considered a nuisance in general agriculture practice, which creates a homogeneous system where a specific part such as higher crop yield is exploited. Planting the same crop every season reduces nutrient in the soil and encourages soil-borne pathogens, which can reduce the crop yield, soil organic matter, soil microbial activity, and lowers nutrient efficiency (Wu et al., 2016; Zhao et al., 2018). Hence, plant diversity is essential part to enhance soil ecosystem functionality (Isbell et al., 2011; Lange et al., 2015) .

No-tillage with weed management system (NTW)

A system that has readily available resources, independent of heavy labor and machinery, can have a positive impact on the current farming systems. Natural farming system (no-tillage with weed mulch (NTW) is being recognized recently by small scale farmers, which was first pioneered by Fukuoka (1978). In this system, weeds are not completely removed from the field, and the above-ground biomass of weeds is slashed with the root still intact in the soil. Slashing the weeds reduces the above-ground competition between the weeds and crops and, at the same time, acts as a mulching resource for the soil. It is depended on locally available resources, self-sufficiency, and conservation agriculture principles. Farmers can grow crops independent of heavy machinery, industrial amendments, and other chemical products. In the NTW system, no herbicides are used to control the weeds. Traditionally, tillage was performed to control the weeds, but weeds have found to be quite beneficial in agricultural lands (Kaneko, 2014) as they can help to avoid yield reduction (Adeux et al., 2019; Głąb and Kulig, 2008) and also act as a resource for soil due to its carbon enhancing properties (Arai et al., 2014) when used as mulch. Researches have also shown that compared with conventional farming, no-tillage with weed has potential to increase soil organic matter, microbial activity and also decrease negative impact in terms of nitrate leaching and global warming potential (Arai et al., 2013; Miura et al., 2013; Yagioka et al., 2015). Hence, NTW might be an appropriate derivative of CA to efficiently use nutrient within the soil ecosystem with reduced N loss to the environment.

Hypotheses

I hypothesize that the weed slashing and mulching NTW practice can conserve the soil organic matter, soil animal activity, and increase nitrogen use efficiency and nitrogen release by using weed mulch.

Impact of tillage on long-term NTW farm

In Chapter 2, I hypothesize that the no-tillage with weed practice can conserve the soil organic matter and soil animal activity by using slash and mulch weed management.

Sustainable N utilization by using weed in NTW

In chapter 3, I hypothesize that slashing and mulching management in NTW can release N to the soil that contributes to crop production.

Nitrogen use efficiency (NUE) in NTW

In chapter 3, I hypothesize that NTW will have higher NUE compared to tillage treatment due to the presence of weeds.

Chapter 2

Short-term impact of tillage on soil carbon and nitrogen in a commercial no-tillage with weed farm

Introduction

The effect of tillage on the soil ecosystem has been well documented throughout the world. Arable soils under a long-term regime of frequent tillage usually suffer from a loss in soil organic matter due to erosion and volatilization, deteriorated soil structure, and destruction of soil animals' habitat, thus reducing agricultural sustainability (Chan, 2001; Kladivko, 2001; Paul et al., 2013). Conversely, conservation agriculture (CA) systems have been repeatedly shown to have a positive impact on soil physical, chemical, and biological properties (Hobbs et al., 2008; Triplett and Dick, 2008). When there is no mechanical disturbance of soil or mixing of soil and residue the actions of ecosystem engineers and the litter transformers such as earthworms and microarthropods, plays an important role for processes like aggregate formation and nutrient cycling (Blouin et al., 2013; Lavelle et al., 2006). The deep, vertical burrows can increase water infiltration and root growth as well as the porosity of the soil that supports both below ground as well as above-ground ecosystems. Their burrowing and foraging activities, decomposing the organic materials, as well as their ability to create different soil structures with specific soil properties changes the soil structure dynamics and the corresponding regulation of soil ecological functions and ecosystem services (Ke et al., 2015; Mutema et al., 2013).

Many long-term studies have compared the change in soil ecosystem in the tillage and no-tillage system (He et al., 2011; Jacobs et al., 2009; Olson et al., 2005). These studies generally establish an experimental field that is initially managed by tillage practice. Usually, these studies take a few years to observe a positive change in soil when transitioning from tillage to no-tillage system. Six et al. 2000 found 38% high carbon and nitrogen concentration in NT treatment in 4 years experiment. Similarly, Balota et al. 2004 showed a 45 % increase C concentration in NT on a 26 years long-term experiment whereas, Oorts et al. 2007 reported an increase of only 10-15% C and N stock in the soil even after 32 years of NT. Brévault et al. 2007 also showed higher abundance and biodiversity of soil macrofauna, three years after the implementation of no-tillage with mulch. Short term studies conducted in 1-2-years of studies also show a similar trend (Astier et al., 2006; Kristensen et al., 2003). These studies, however, show the changes in soil from a tillage site, which gets converted into a no-tillage site. There are very few studies that show the immediate short-term change in the soil ecosystem from tillage to the no-tillage system. The above studies show that it takes a long time and resources to transition from tillage to no-tillage system. But, tracing the changes in the soil from no-tillage to tillage can show the intensity of tillage impact on the soil ecosystem. In this experiment, the short-term impact of tillage on commercial no-tillage with weed (NTW), which has been managed for 30 years, was evaluated, particularly on soil carbon and nitrogen concentration and soil macrofauna activity.

Many researchers have demonstrated that Conservation Agriculture (CA) is effective in improving soil physical and chemical properties (Mloza-Banda et al., 2016; Parihar et al., 2016), crop yields and reducing energy required and production cost (Brévault et al., 2007; Mbuthia et al., 2015; Tullberg et al., 2007). No-tillage with weed mulch (NTW) (Arai et al., 2013; Yagioka et al., 2015) is a type of CA system that has been recently recognized by small-scale farmers, although it was first pioneered by Fukuoka (2009) in the year 1978. In this system, naturally occurring weeds in an agriculture field are grown with the crops throughout most of the crop period, creating a highly diverse plant ecosystem that can potentially be quite beneficial in agricultural lands (Kaneko, 2014). In this system, weeds are not completely removed from the field; the aboveground biomass of weeds is slashed without disturbing the roots. Slashing the weeds not only reduces the aboveground competition between the weeds and crops but it also acts as a green mulch. A closed nutrient cycling can be developed in NTW without any external inputs and the presence of weeds can reduce the nutrient loss to the environment by capturing the available N in the soil (Yagioka et al. 2015). In this study, short-term impact of tillage on a commercial no-tillage with weed mulch system was investigated. I hypothesize that larger soil animals, litter and roots will be immediately reduced due to tillage subsequently reducing the soil carbon and nitrogen concentration.

Materials and method

Study site

The study site is located in Shinshiro City, Aichi Prefecture, Japan (34° 52' 45.93" N and 137° 33' 8.4672" E). The soil is Andic soil of pelitic schist origin (Soil Classification System of Japan, 2017). The study site is a commercial long-term no-tillage farm. The site has been managed with no-tillage treatment for 30 years (Mr. Matsuzawa, personal communication). The farm is managed without the application of fertilizer and pesticides; instead, weeds are used as the only source of input. The weeds (Mostly Italian ryegrass) that are growing naturally in the farm are rolled overusing a barrel and then slashed using a hammer knife mower. The slashed weeds are mulched on the soil surface.

Experimental design

Two treatments, i.e., tillage and no-tillage, were set in October 2017. Before this establishment, the whole farm was managed by no-tillage. The site consisted of 5 blocks; each block was divided into 2 adjacent plots (a total of 10 plots), with each plot measuring 0.25 m^2. The tillage plot was disturbed up to 30 cm depth by using a shovel. The no-tillage plot was not disturbed, and the above-ground biomass of the weeds was slashed and recovered as weed samples. Our study did not measure any crop yield; rather, our aim was to evaluate the soil change after introducing tillage on a no-tillage farm.

Soil sampling and analysis

The initial soil samples were collected from three different depths (0–5 cm, 5–10 cm and 15–20 cm) using a 100 cm^3 core in October 2017, before the establishment of tillage and no-tillage plots. The final soil sample was collected in February 2018 form tillage and no-tillage plots. The weight of soil samples was measured, after which they were oven-dried at 105°C for 24 hours. These samples were used to calculate soil physical and chemical properties such as pH, EC (electrical conductivity), bulk density, water content, and C and N concentration. The pH and EC in soil and water solutions (1:5) using a pH/EC meter (pH/COND METER HORIBA, Kyoto, Japan). Total carbon and total nitrogen content were analyzed using a Macro Coder JM1000CN (J-Science Lab Co., Ltd., Kyoto, Japan). Carbon and nitrogen change were calculated by subtracting the final C and N content of tillage and no-tillage treatment with initial C and N content of our study site.

Soil macrofauna and root sampling

The soil macrofauna and roots were collected from 25 x 25 cm quadrats by hand sorting to a depth of 20 cm in June 2018. The macrofauna was collected using forceps and preserved in 80% alcohol, and the biomass of the taxonomic group was measured. The roots were separated from the soil with the use of a sieve with a 0.5 mm mesh size. The roots were washed, and collected roots were oven-dried at 45°C for 2 days, and their dry weight was measured.

Weeds, surface litter, and root biomass

Weed sampling was performed on 50 cm x 50 cm quadrats using shears. The sampled weeds were oven-dried at 45°C for 3 days, then the dry weight was measured. Similarly, surface litter was also sampled in the same quadrat, which was hand-picked. The sampled litter were oven-dried at 45°C for 2 days, then the dry weight was measured. The samples were collected four times in September 2017, February 2018, June 2018, and October 2018.

Nitrogen leaching

Ion exchange resin (IER, mixed anion, and cation, Amberlite MB-1, ORGANO, Tokyo, Japan) was assembled in a circular disk made from PVC pipe (outer diameter 6.0 cm, inner diameter 5.1 cm, height 1.0 cm) with one side covered in nylon mesh. 25 ml (12 g dry weight) IER was filled in the disk and inserted in a small bag made of a stocking. The prepared resin bag was then buried at a depth of 10 and 30 cm depth in each plot. The bags in no-till were inserted from the periphery of the plot, so the soil structure on the plot was not disturbed. A string was then attached to the bag to determine the location. The bags were inserted in September 2017 and retrieved in June 2018. The bags were replaced with new resin bags and were extracted in October 2018. Upon recovery, the IER was dried for over 24 hours at 40°C, after which the dry weight was measured. The sample was then stored in the freezer at -10°C until it was subjected to inorganic nitrogen analysis. For measurement of inorganic nitrogen, 2.5 mg dry resin was measured in a flask, after which 25 ml 2 N KCl was added and shaken for 1 hour at 170 rpm. The solution sample was then filtered, and ammonium nitrogen (NH_4-N) and nitrate-nitrogen (NO_3-N) were extracted with stock solutions (same concentration as 2N KCl). The analysis was performed using an autoanalyzer (Future, Alliance Instruments, Frépillon, France).

Litter decomposition

A polyester bag of 24 cm x 32 cm area with mesh size 1 mm was used as a litter bag. *Imperata* *cylindrica* was used as a model green litter because it is a perennial plant and is abundant in temperate and tropical areas of the world because of its rapid recovery from disturbance and its competitive nature (Chikoye et al., 2000; Tollens et al., 2013). Leaves of *I. cylindrica* were collected with the help of shears that were available around the study site. The litter bag was filled with 20 g (fresh weight) biomass that was cut to fit. One more litter bag with mixed litter was placed on no-tillage site to replicate the general management practice of our study site. Litterbag containing *I. cylindrica* was placed in both tillage and no-tillage plots, and an additional one litter bag containing mix litter was placed in no-tillage treatment (total 3 litter bags in each block). The litter bag was placed in September 2017 and was retrieved in February 2018 (5 months duration).

Statistical analysis

All statistical analysis was performed using the statistical program R version 3.4.3 (R Core Team, 2018). The effects of treatments on weed, litter, root biomass, and N releases from litter and weeds were analysed by analysis of variance (ANOVA), where the response variables were the weed, litter, root biomass and N releases and the explanatory variable was the tillage treatments. Similarly, the soil physio-chemical properties and total soil macrofauna biomass were also analyzed using ANOVA between the two treatments. When a significant effect in ANOVA was observed ($p < 0.05$), a significant difference between pairs of means were tested through Tukey's HSD test. In all cases, significance was set at $p<0.05$.

Results

Soil analysis

Soil pH was similar between tillage and no-tillage treatments and was identical to the pH of the initial soil sample (Table 1). Soil EC remained similar in no-tillage and initial soil samples, but tillage treatment showed a decreasing trend. However, statistical significance was not observed. Soil carbon concentration significantly decreased after the implementation of the tillage treatment. Nitrogen also showed a decreasing trend after the tillage; however, statistical significance was not observed. Compare to the initial sample; bulk density increased in both treatment but without any significant difference.

Soil macrofauna

Almost all soil animals showed a reduction in number after the introduction of tillage. The total biomass of soil animals was significantly higher in no-tillage plots compare to tillage plots. From those soil animals, only earthworm biomass was significantly reduced after the tillage ($P < 0.05$) (Table 2). Other soil animals, including Araneae, Hexapods, and Diplopoda, were reduced after the tillage but were not significant.

Weed, litter and root biomass

In February 2018, during winter, weeds on tillage plot showed significantly lower biomass compared to no-tillage treatment. During September, June, and October, the weeds biomass was similar in both treatments. Soil surface litter biomass was significantly affected immediately after the tillage. In September, the litter biomass was significantly lower in tillage compared to no-tillage plot. Other sampling periods showed a decrease in the litter biomass on the tillage plot, but a significant difference was not observed. Roots showed significantly lower biomass in June sampling on tillage treatment compared to no-tillage plots. Other sampling periods showed a decreasing trend of root biomass on tillage sites; however, a significant difference was not observed (Table 3).

Nitrogen leaching

Nitrogen leaching was measured during two intervals, i.e., from September 2017 to June 2018 and June 2018 to October 2018. Nitrogen leaching did not show any significant difference between the two tillage treatments, measured at two depths at 0-10 cm and 0-30 cm (Table 4). Similar nitrate and ammonium amount were observed in both sampling periods. However, an increasing trend of ammonium and nitrate amount was observed in the tillage treatment.

Litter decomposition and N release

Imperata nitrogen concentration was significantly lower after 6 months on the field on no-tillage treatment. Tillage treatment did not have any significant change in N concentration and N amount. Mixed litter placed on no-tillage treatment showed a significant change in N amount, but N concentration was not affected. The nitrogen released from *I. cylindrica* was lower in both no-tillage (68.3 mg/m^2) and tillage (26.9 mg/m^2) treatments compared to mixed litter in no-tillage (1173.2 mg/m^2). Similarly, N release from weeds was estimated using *I. cylindrica* N release were 167.2 mg/m^2 and 56.70 mg/m^2 in no-tillage and tillage plot, respectively. Whereas N release estimated from chopped mixed weed was 2091 mg/m^2 for no-tillage.

Discussion

Weeds

Generally, tillage is implemented in agricultural land to control the weeds, but our results showed that tillage did not have a significant impact on weed biomass. Weed biomass was similar in both tillage and no-tillage plots. Tillage eliminates the crop residue, which is a suppressor of weed establishment. Crop residues suppress weed establishment by altering environmental conditions, physical impeding seedling growth, or inhibiting germination and growth by allelopathy (Anderson, 2004). Our study showed only a 1-year trend of weed on the two-tillage system. But this result was similar to other long-term studies indicating that weed diversity and biomass were not influenced by tillage intensity (Demjanova et al., 2009; Plaza et al., 2011). In our study, the plots managed by NTW did not completely control the weeds either. Studies have shown that conservation agriculture systems did not reduce the density of perennial and annual weeds and sometimes may not be able to control the well-established perennial weeds (Carr et al., 2013; Demjanova et al., 2009). Our study site was covered with Italian ryegrass, which is a fast-growing perennial weed. The weeds were managed in no-tillage plot by manually slashing the weeds; however, the management practice of the farm is done by using a hammer mower, which is a more effective weed management practice on this particular farm.

The shoot to root ratio gradually increased and was higher in tillage treatment compared to no-tillage treatment at the end of the experiment. This suggests that the root development was relatively poor in tillage treatment. Tillage can modify certain soil properties such as bulk density and aggregate stability and may have a significant impact on root growth, as well as give rise to differences in soil nutrient status. A major part of soil carbon content is stored as the root and also act as food resources for soil animals such as earthworms (Katterer, 2011; Springett and Gray, 1997). In our study, tillage treatment consisted of manual tilling of land up to 20 cm. During this treatment, the roots, as well as other organic matter, are removed or mixed in the soil. However, in no-tillage treatment, the roots were not disturbed throughout the experiment, which conserved and promoted the root growth.

Litter decomposition

Soil surface residue improves soil structural properties and conserves water and protects the soil against water and wind erosion. In our study, a decrease in litter biomass was observed after the introduction of tillage compared to no-tillage plots. A significant decrease in litter biomass was observed immediately after the tillage. Tillage reduces the surface residue and disrupts soil structure, accelerating surface runoff, and soil erosion. Hence, surface residue and organic matter were instantly affected by tillage, and a decreasing trend was observed throughout the experiment.

Soil macrofauna

In our study, soil animals including, Hexapoda, Diplopoda, and Araneae, showed reduced activity after tillage was introduced while earthworm biomass decreased by two folds. Less soil disturbance and the presence of weed mulch or litter residue in NTW may also have influenced the soil fauna. Availability or increasing the soil surface residue rate has shown to have significant positive effects on soil macrofauna abundance (Mutema et al., 2013). Similarly, a combination of no-tillage and soil cover can provide conditions that favor soil fauna activity, starting with relatively big-sized organic material primary shredders, followed or accompanied by progressively small-sized fauna groups as the decomposition process becomes more complex. The larger soil animals like earthworms are responsible for propagating pores, which give soils their characteristic open structures to facilitate air circulation, water infiltration, and root development, which is why earthworms are the immediate recipient of tillage impacts. A positive effect of earthworm on the aboveground plant growth was found to be twice as high in no-tillage soil compared to tillage soil (Van Groenigen et al., 2014). Undisturbed soil retains the soil nutrient, and earthworms stimulate plant growth by mineralization of the available nutrient in the soil (Spurgeon et al., 2014). A review by (Chan, 2001) reported that tillage could change the earthworm abundance (by 2–9 times) as well as the composition of the species. However, most of these researches were long term studies. Here, we show the immediate impact of tillage, which significantly reduced the earthworm biomass within one year.

Soil carbon and nitrogen change

Carbon change was indirectly affected by earthworm biomass, which is analyzed together and showed in Fig. 1. A positive correlation between earthworm and bulk density of soil was observed. Bulk density slightly increased in both tillage and no-tillage samples compared to the initial soil sample. A meta-analysis by Lang and Russell 2019 found that earthworm effects on bulk density depended on the specific species, soil texture, and earthworm body mass, where the bulk density was highly variable and ranged from a reduction of bulk density by 25% to an increase of bulk density by 36%. The significant impact of earthworm on bulk density was not observed in this study; however, a slight increase in bulk was observed both in tillage and no-tillage treatment. Although bulk density has a relatively low degree of spatial variation (Grossman and Reinsch, 2002), some studies have shown bulk density to change with time due to factors such as water content, soil animal activity, and root growth (Logsdon and Cambardella, 2000).

Our study showed a positive correlation between earthworm and roots. Earthworms modify soil profiles by burrowing, moving particles within and between horizons, forming aggregates, and changing porosity, aeration that helps in the growth and development of the roots. It was estimated that root C contributed to 2.69 Pg yr^{-1} of the global soil C pool, which can have a major impact on the soil carbon cycle (Robinson, 2007). Litter also positively correlated with earthworm biomass. An increase in soil cover and organic matters increases the soil animal activity as it supplies food and establishes a suitable habitat. No-tillage treatment had higher litter compared to tillage that can attract earthworms and other soil organisms to break down the organic matter and helps in releasing nutrients back to the soil.

Here, earthworm indirectly affected the soil carbon change through bulk density and root and litter availability. Reduction in earthworm biomass on tillage treatment significantly reduced the soil carbon pool.

Unlike soil carbon change, nitrogen change was similar in both treatments. Earthworm showed a positive correlation with roots, litter, and total nitrogen release (Fig.2). Suggesting that earthworm facilitated the growth of roots, litter utilization leading to nitrogen release. But there was a negative correlation between roots and litter as well as roots and nitrogen release. In the NTW system, only the above-ground part of weeds is slashed, conserving the roots. The roots of weeds can reuse the nitrogen released by the mulched litter and other organic matter for regrowth, reducing the amount of N in the soil. This negative correlation with roots and litter and nitrogen release might have some contribution to the uniform condition of soil nitrogen. Tillage generally has been showed to degrade the soil through erosion of soil and organic matter resulting in a reduction in total soil N (Wright and Hons, 2005). But our study covered short-term change on the soil, which did not show significant N reduction. Hence, in our short-term study, a significant reduction in soil carbon concentration after the tillage treatment was observed, while soil nitrogen concentration remained similar between both treatments.

Conclusion

Establishing a stable and sustainable soil ecosystem through conservation agriculture and other agroecological approach is a long-term process. During this transition period, many challenges, including low production, weed, and pest control, need to be continuously observed and managed. Researches have shown that transitioning from conventional tillage agriculture system to no-tillage conservation agriculture system can take from 5 to10 years. But here, I show the severity and immediate impact of tillage on the no-tillage farm. Our site was managed using no-tillage practice for 30 years, and the impact of tillage on soil ecosystem was immediately seen within one year. A substantial amount of time and resources is required to transition to the no-tillage system, but disruption through tillage is an instantaneous process and shows the severity of the tillage system on the soil ecosystem.

Table 1. Comparison of soil physical and chemical parameters of Initial soil condition of the farm and no-tillage with weed (NTW) and tillage experimental plot. Means with different letters are significantly different at $p < 0.05$ (Tukey's HSD test). ΔC and ΔN (g/m^2) represent carbon and nitrogen change, respectively.

	pH				EC (mS/m)				Bulk density (g/m^3)				Carbon (%)				Nitrogen (%)				Carbon (g/m^2)				Nitrogen g/m^2)				ΔC	ΔN
	Mean	±	S.D.		Mean	±	S.D.		Mean	±	S.D.		Mean	±	S.D.		Mean	±	S.D.		Mean	±	S.D.		Mean	±	S.D.			
Initial	6.23	±	0.06	a	7.25	±	1.4	a	0.69	±	0.02	a	5.57	±	0.6	a	0.55	±	0.03	a	1894	±	149	a	189	±	11.3	a		
NTW	6.19	±	0.03	a	8.14	±	2.2	a	0.80	±	0.03	a	5.25	±	0.3	a	0.49	±	0.01	a	2072	±	78	a	198	±	5.5	a	178	10
Tillage	6.09	±	0.06	a	5.15	±	0.8	a	0.86	±	0.03	a	4.44	±	0.5	b	0.44	±	0.03	a	1836	±	145	a	184	±	9.1	a	-58	-5

Table 2. Biomass (g/m^2) of different soil organisms in no-tillage with weed (NTW) and tillage plots. Earthworm biomass was significantly reduced after the introduction of tillage. ANOVA($P<0.05$)

	NTW				Tillage			
Soil animals	Mean	±	S.D.		Mean	±	S.D.	
Amphipoda	0.03	±	0.01	a	-		-	a
Ant	0.06	±	0.01	a	0	±	0	a
Aranea	0.53	±	0.19	a	0.25	±	0.09	a
Chilopoda	-	±	-	a	0	±	0	a
Hexapoda	1.5	±	0.37	a	0.06	±	0.04	a
Dermaptera	0.1	±	0.03	a	0.03	±	0.01	a
Diplopoda	1.44	±	0.68	a	0.43	±	0.2	a
Gastropoda	0.18	±	0.1	a	-		-	a
Gryl	0.01	±	0.01	a	-		-	a
Hymenoptera	0.08	±	0.05	a	0.05	±	0.03	a
Isopoda	0.27	±	0.1	a	-		-	a
Earthworm	22.58	±	5.97	a	3.87	±	0.79	b
Total	26.78		1.69	a	4.69		0.27	b

Table 3. Litter, roots, and weed biomass (g/m^2) on no-tillage with weed (NTW) and tillage treatment. Samples were collected four times in September 2017, February, June, and October 2018. Means with different letters are significantly different at $p<0.05$ (Anova).

Date	Treatment	Litter				Roots				Weeds (Shoot)				Shoot: Root			
		Mean	±	S. D.		Mean	±	S. D.		Mean	±	S. D.		Mean	±	S. D.	
17-Sep	NTW	55.66	±	10.14	b	5.25	±	1.15	a	103.58	±	25.22	a	141.65	±	48.70	a
	Till	16.93	±	3.30	a	2.08	±	0.84	a	62.82	±	13.29	a	81.05	±	17.65	a
18-Feb	NTW	46.37	±	18.09	a	103.58	±	25.22	a	74.19	±	11.76	b	1.26	±	0.39	a
	Till	7.18	±	3.52	a	62.82	±	13.29	a	16.93	±	3.30	a	0.82	±	0.27	a
18-Jun	NTW	77.54	±	20.85	a	96.55	±	9.45	b	208.77	±	23.80	a	2.28	±	0.19	a
	Till	40.84	±	10.65	a	58.23	±	5.70	a	166.22	±	24.35	a	2.93	±	0.37	a
18-Oct	NTW	62.59	±	8.56	a	41.42	±	13.21	a	192.32	±	36.80	a	11.50	±	4.03	a
	Till	35.62	±	10.52	a	20.40	±	3.81	a	218.42	±	61.90	a	20.72	±	6.07	a

Table 4. Ammonium and nitrate (g/m^2) in no-tillage (NTW) and tillage treatment at two depths (10 cm and 30 cm). No significant change was observed between the treatments both in ammonium and nitrate amount at two depths.

Treatment	Depth (cm)	Ammonium			Nitrate		
		Mean	±	S. D.	Mean	±	S. D.
No tillage	10	0.37	±	0.02	1.88	±	0.11
	30	0.49	±	0.03	2.22	±	0.10
Tillage	10	0.53	±	0.04	1.89	±	0.12
	30	0.69	±	0.05	2.41	±	0.08

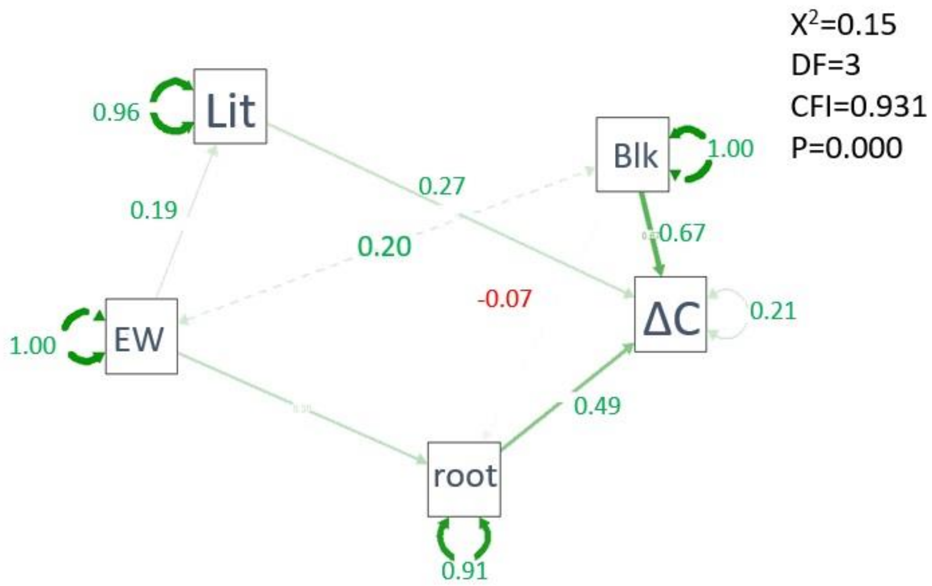

Figure 1. Structural equation model showing the effect of earthworm on carbon change. Green single head arrow and numbers show positive regression, and the double head arrow shows correlation. Red arrow and number show negative regression.

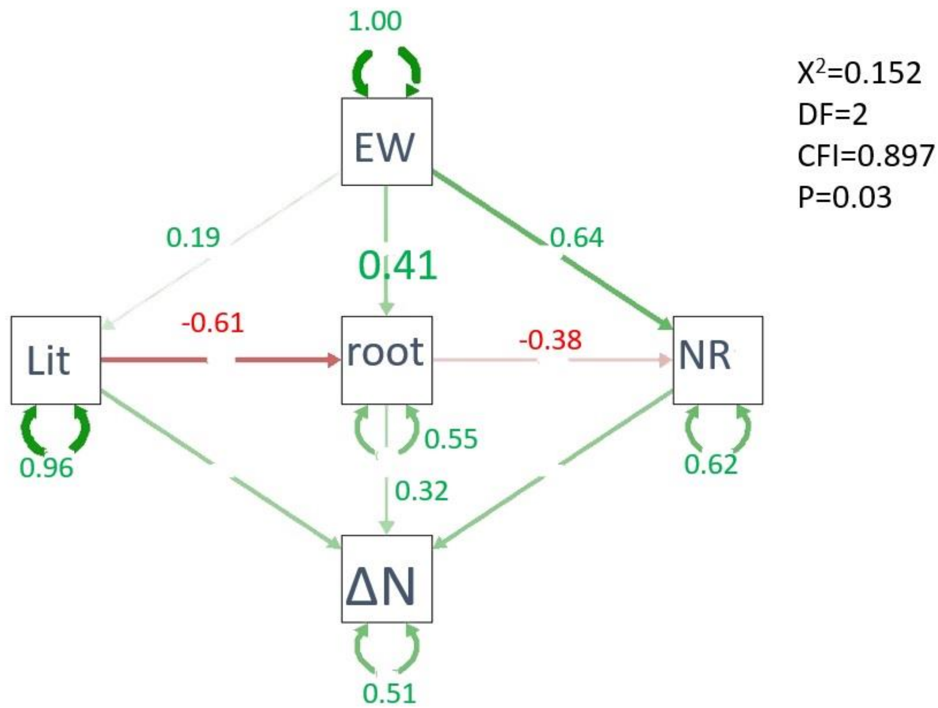

Figure 2. Structural equation model showing the effect of earthworm on nitrogen change. Green single head arrow and numbers show positive regression, and the double head arrow shows correlation. Red arrow and number show negative regression.

Chapter 3

Use of weed as a nitrogen resouce with slashing and mulching management in no-tillage with soybean

Introduction

Agricultural lands cover about 11% (1.5 billion ha) of the earth's land surface (FAO, 2003), of which 84% is owned by small-scale farmers (FAO, 2014; Lowder et al., 2016). Besides producing food and commodities, these lands also possess various multi-functionalities like social, economic, and environmental functions (Van Huylenbroeck et al., 2007). Increasing pressure on agricultural lands, water, and other resources to produce more food is imminent with the exponential growth of the world population, which is expected to reach 9.1 billion by 2050 (FAO 2014). During the last century, food production kept up with the growing population by the introduction of the agricultural green revolution (GR) (Tilman, 1998). The fundamental practice of GR consists of the introduction of the first generation of inputs (fertilizer and pesticides) and high yield variety crops that significantly increased production over a period of 25 years from 1965 to 1990 (Hazell 2010). GR was able to achieve its goal of food production and ensure global long-term food security. However, GR has been criticized because of its unexpected repercussions in water use, soil degradation, and chemical runoff, which have led to serious environmental impacts beyond the designated areas (Funabashi 2018). These environmental consequences are widely recognized as a potential threat to the long-term sustainability and expansion of the GR (Lynch 2007). However, GR technology is not directly responsible for the above-mentioned issues; rather, the responsibility lies with the unregulated use of industrial amendments and extension of practices into areas such as hilly regions that could not sustain such high levels of intensification. Because the GR strategy was based on intensification of selected and specific areas, its contribution to challenging landscapes and poverty dominated areas was relatively low (Pingali 2012).

Conservation agriculture (CA) is suitable for small-scale farms representing a combination of agronomic technologies that include minimum disturbance of soil, conservation of soil by mulching with residues and cover crops, and diversification of cropping systems (Lalani et al., 2016). The most important principle of CA is that it can be applied to all agricultural landscapes and can be adapted with local practices (FAO, 2008). CA is one of the most lucrative and environmentally sound agroecosystems; in recent years, CA has been adopted over 125 million ha of cropland (9% of global arable land) across different parts of the world (Kassam et al., 2009). However, site-specific factors like soil type, topography, climate, and their combination, and management practices bring various challenges to the table (Scopel et al., 2013). Hence before the adoption of CA, stakeholders should acknowledge their production objectives, the costs and risks of CA, and other technical aspects (Giller et al., 2009). The above aspects should be carefully studied to successfully adopt CA, which is complicated to learn and implement; therefore, new adopters often encounter obstacles like weeds and pest control before reaping its full advantages (Derpsch et al., 2010).

One of the major challenges is the weed outbreak, and it is considered a major reason why farmers oppose CA (Derpsch et al., 2010; Giller et al., 2009). Weeds can be a serious problem in agriculture as they compete with crops for land, water, nutrients, and other resources resulting in low crop yield. Hence, it requires site-specific integrated approaches for effective weed management such as biological weed control, nutrient management, and modified tillage practice. Successful weed management does not necessarily mean total control of weeds on a crop field, rather developing a system that reduces weed competition with crops. Numerous cultural or local management practices are vital in the sense that they are appropriate for certain types of climate and landscape. The natural farming system (no-tillage with weed mulch; NTW) (Arai et al., 2013; Yagioka et al., 2015) has been recently recognized by small-scale farmers, although it was first pioneered by Fukuoka (1978). In this system, crops and weeds grow together throughout most of the crop period, creating a highly diverse plant ecosystem that can potentially be quite beneficial in agricultural lands (Kaneko, 2014). In this system, weeds are not completely removed from the field; the aboveground biomass of weeds is slashed without disturbing the root (Fig. 1). Slashing the weeds not only reduces the aboveground competition between the weeds and crops, but it also acts as a green mulch. A closed nutrient cycle can be developed in NTW without external inputs, and the presence of weeds can reduce the nutrient loss to the environment by capturing the available nitrogen (N) in the soil (Yagioka et al. 2015).

Weeds are generally considered as a detrimental factor in agricultural land; however, they can also be a source of nutrients when used as a mulch, and they are freely available in the field. The rate of N release from any kind of mulch depends on the decomposition process, which is responsible for breaking down dead organic matter and mineralization of N and other minerals to the soil. During this process, structural compounds accumulate, and soluble nutrients can be mineralized or immobilized depending on the litter quality and its surrounding biotic and abiotic components (Cotrufo et al., 2010). Hence, litter quality is an important factor for decomposition. For instance, green litter contained twice or higher the concentration of N when compared with brown litter, which resulted in a high degree of degradation (Sanaullah et al., 2012). Soil fauna also controls litter decomposition, mostly by consuming the detritus with the help of microorganisms, which degrade and metabolize the complex organic matter (Handa et al. 2014).

Previous studies have investigated the effect of organic residues in the no-tillage agriculture system by demonstrating increased soil organic matter and crop yield (Nascente et al. 2013). However, the mulch resources used were generally an external resource. Living mulch and cover crops are also used to improve the soil and, most importantly, control the weeds (Campiglia et al. 2010). But here, weeds present within the field were used for mulching that reduces the time and resources to manage the cover crops. For the determination of the appropriate weed control method in NTW, the weeds were slashed with three different frequencies, and N release by slashed weeds was calculated. The objective of slashing weeds was to control the competition between weeds and crops and simultaneously use weeds as an input resource to the soil. I hypothesized that, with the increasing frequency of weed slash, N release from the slashed weed increases, resulting in high soil N pool due to retention of N in the soil pool, eventually increasing the crop yield.

Method

Site description

The study site is located in the Kurokawa Science Field Centre of Meiji University (35°36'32"N, 139°27'11"E, elevation 118 m above sea level) in Kawasaki, Kanagawa Prefecture, Japan. The annual precipitation and average temperature were 1506 mm and 15.7°C, respectively. The soil was volcanic black Andisol. The study site was managed as a no-tillage farm for 4 years from 2012 to 2016. The experiment was carried out using randomized block design, which consists of 4 random blocks; each block was divided into 3 treatments (a total of 12 plots), with each plot measuring 10 m x 4.2 m. Each plot was further divided into 3 rows with dimensions of approx. 10 m x 1.4 m. Soybean (Tsukui Zairai) was planted on 22 June 2017 and was harvested on 6 November 2017.

The plots were slashed zero (S0), once (S1), and twice (S2). The weed management was done by slashing the aboveground biomass of the weeds using sickle without disturbing the roots. The weeds were slashed in all the three treatments before seeding in June to provide a consistent starting point. After the initial slash, the weeds were not slashed in the S0 treatment and grew with the crops throughout the crop season. The aboveground part of weeds in treatments S1 and S2 were slashed on 26 July, and the weeds in treatment S2 were slashed once more on 31 August 2017. The weeds were the only input given to the soil in this study, and no fertilizer or herbicides were used.

Soybean yield and weed sampling

The soybeans were harvested and sorted according to the plot, and then the beans were collected. The samples were air-dried using an oven dryer at 45°C for 2 days. The grain biomass of soybean was measured for the yield data. The samples were ground and used for C and N analysis. Weed sampling was performed using 100 cm x 50 cm quadrats in June, July, August, and October. The sampled weeds were oven-dried at 45°C for 3 days. The dry weight of weed was measured and was identified for the determination of weed community structure.

N release from litter decomposition and slashed weed

A polyester bag of 24 cm x 32 cm area with mesh size 1 mm was used as a litter bag. *Imperata cylindrica* was used as a model green litter because it is a perennial plant and is abundant in temperate and tropical areas of the world because of its rapid recovery from disturbance and its competitive nature (Fang et al. 2007). Fresh *I. cylindrica* was collected with the help of shears. The litter bag was filled with 20 g (fresh weight) biomass that was cut to fit. Three litter bags were placed in each plot in a randomized manner on the soil surface in June 2017 and were retrieved in July, August, and October (i.e., 12 bags per month). Furthermore, one litter bag each was replaced in July and August and was retrieved in August and October, respectively, to measure the decomposition after the first and second slashing. The five periods of the litter bag measurements were as follows: June–July, June–August, June–October, July–August, and August–October.

The total N release from litter to the soil in each treatment was calculated by subtracting the final N content from the initial N content of litter from June to October. The initial slash was done in June, and the release was calculated by adding N release from June-July, July-August, and August-October in all three treatments. The first slash was done in treatment S1 and S2 in July, and an additional litterbag was added in each treatment to represent the slash weeds. Hence, for the evaluation, the nitrogen release from the first slash, N release from July-August, and August-October were measured. The decomposition between August and October of litter set in July was not measured so, the estimation of N release from weeds for S1 and S2 will be an underestimate. Lastly, the second slash was done only in treatment S2 in August, and N release from a new litter bag representing the slashed weed was measured from August-October.

The N release from litter bag and weeds during an interval was calculated using the following equations:

$$\text{Litter bag N release } (t_0 - t_1) = (N_0i * W_0i) - (N_1i * W_1i) \quad (1)$$

$$\text{Weed N release } (t_0 - t_1) = (W_0i / W_1i * W_0w) * N_0i - W_0w * N_1i \quad (2)$$

where N_xi = N concentration (%) of *I. cylindrica* in a litterbag at time x, W_xi = dry weight (g/m^2) of *I. cylindrica* in a litterbag at time x, and W_0w = The initial dry weight (g/ m^2) of weeds in a litter bag. The amount of N released from slashed weed was estimated by using the litter bag N concentration, and the amount of weed biomass slashed, assuming that the N contents and N released to the soil were similar between *I. cylindrica* and the weeds, which were composed of multiple species.

The decomposition rate of the litterbag was calculated by fitting the following equation (Adair et al. 2008):

$$W_{t1}=W_{t0}e^{-k} \quad (3)$$

where W_{t1} is the mass of litter at a certain time; W_{t0} is the initial mass of litter; e is the base of the natural logarithm; k is the decomposition constant, and t is the amount of time passed since the initial measurement.

Soil sampling and analysis

Soil samples were collected twice in June and November 2017, using an auger (Daiki, DIK 110-C, Daiki Rika Kogyo Co. Ltd, Saitama, Japan) of 5 cm diameter with a 30 cm soil depth at each plot. The collected samples were stored in a cylindrical tube at −4°C for a day before processing. The 0-10 cm soil was separated, and a part of the soil sample was weighed and then oven-dried at 105°C for 24 h to measure soil physical and chemical properties: bulk density, water content, pH, electrical conductivity (EC) and C and N concentrations. Soil pH and EC were measured by pH/EC meter (pH/COND METER, HORIBA, Kyoto, Japan) in a soil: water solution (1:5). Analysis of total C and total N content was performed with Macro Coder (JM1000CN, J-Science Lab Co. Ltd, Kyoto, Japan). A subsample of 0–10 cm was separated to estimate microbial biomass.

Root analysis

The roots were collected from 25 x 25 cm quadrats by hand sorting to a depth of 20 cm. The roots were separated from the soil with the use of a sieve with a 0.5 mm mesh size. The roots were washed, and the crop roots and weed roots were separated using forceps. The collected roots were oven-dried at 45°C for 2 days, and their dry weight was measured.

Nitrogen leaching

Ion exchange resin (IER, mixed anion, and cation, Amberlite MB-1, ORGANO, Tokyo, Japan) was assembled in a circular disk made from PVC pipe (outer diameter 6.0 cm, inner diameter 5.1 cm, height 1.0 cm) with one side covered in nylon mesh. 25 ml (12 g dry weight) IER was filled in the disk and inserted in a small bag made of a stocking. The prepared resin bag was then buried at a depth of 10 and 30 cm depth in each plot. The bags in no-till were inserted from the periphery of the plot, so the soil structure on the plot was not disturbed. A string was then attached to the bag to determine the location. The bags were inserted in September 2017 and retrieved in June 2018. The bags were replaced with new resin bags and were extracted in October 2018. Upon recovery, the IER was dried for over 24 hours at 40°C, after which the dry weight was measured. The sample was then stored in the freezer at -10°C until it was subjected to inorganic nitrogen analysis. For measurement of inorganic nitrogen, 2.5 mg dry resin was measured in a flask, after which 25 ml 2 N KCl was added and shaken for 1 hour at 170 rpm. The solution sample was then filtered, and ammonium nitrogen (NH_4-N) and nitrate-nitrogen (NO_3-N) were extracted with stock solutions (same concentration as 2N KCl). The analysis was performed using an autoanalyzer (Future, Alliance Instruments, Frépillon, France).

Microbial biomass

Phospholipid fatty acid (PLFA) analysis was used to determine the microbial biomass in the soil (0–10 cm). The samples were stored at −20°C until the PLFAs were extracted from the soil. Both initial (June) and final (November) soil samples were analyzed to observe the effect of slash and mulch treatment on soil microbial biomass. PLFAs were extracted from 1 g finely ground freeze-dried samples using a procedure based on Frostegård et al. (2010) and Ichihara and Fukubayashi (2009). Lipids were extracted with one-phase chloroform-methanol–phosphate buffer, and the PLFA fraction was separated using silicic acid columns (BOND ELUT LRC-SI; Varian, Palo Alto, CA, USA) before trans-esterification with mild alkali and a final uptake in dichloromethane. Methyl nonadecanoate (19:0) was added to each sample as an internal standard to quantify the peak areas. The fatty acid methyl esters were separated by gas chromatography and identified with a Sherlock Microbial Identification System

(MIDI, Newark, DE, USA). The fatty acids 15:0iso, 15:0anteiso, 16:0iso, 16:0 10-methyl, 17:0iso, 17:0anteiso, 17:0 10-methyl and 18:0 10-methyl were used to estimate gram-positive bacterial biomass; 16:1ω7c,17:0cyclo, 18:1ω7c and 19:0cycloω8c were used to estimate gram-negative bacterial biomass; and 16:1ω5c and 18:2ω6,9 to determine fungal biomass (Frostegård et al. 2010).

Statistical analysis

All statistical analysis was performed using the statistical program R version 3.4.3 (R Core Team, 2018). The effects of treatments on soybean, weed biomass and N releases from litter and weeds were analyzed by one-way analysis of variance (ANOVA), where the response variables were the soybean, weed biomass and N releases and the explanatory variable was the slashing treatments. Similarly, the soil physio-chemical properties and total soil microbial biomass in the initial and final stages were also analyzed using one-way ANOVA among the three treatments. When a significant effect in ANOVA was observed ($p < 0.05$), a significant difference between pairs of means were tested through Tukey's HSD test. In all cases, significance was set at $p < 0.05$. Permutational multivariate analysis of variance (PERMANOVA) was used to evaluate the change in weed and soil microbial community structure in the three treatments using the *vegan* package in R for each sampling time. Repeated measures ANOVA was performed to compare changes in weed biomass in June, July, August, and October among the three treatments. The changes in initial and final soil microbial biomass among the treatments were also compared using repeated-measures ANOVA.

Results

Crop and weed biomass

The slashing frequency significantly affected the total yield of soybean (Table 1), where both

S1 and S2 were significantly higher than S0 ($F = 9.748$, $Df = 2$, $p < 0.05$). Weed biomass also showed a significant difference in October ($F = 2.83$, $Df = 3$, $p = 0.05$), where S2 had significantly lower biomass than that of S0 and S1. The sum of weed biomass that was slashed and used as mulch was 90 g/m^2, 196 g/m^{2,} and 295 g/m^2 in treatments S0, S1, and S2, respectively. The weed community structure is shown in Table 2. PERMANOVA analysis showed a marginal difference ($p = 0.06$) in the weed community structure among the three treatments in October at the end of the experiment (Fig 2). The weeds species sampled in July and August did not show any changes among the three treatments. The root biomass of soybean and weeds showed an increasing trend with the increase in slashing frequency, but a statistical difference was not observed among the three treatments ($F = 0.47$, $Df = 2$, $p = 0.6$).

Litter decomposition and N release

Dry weight, C and N concentration of *I. cylindrica*, which was used in the litterbag, was not different at each setting of the litter (Table 3). Litter moisture content was almost two times higher in treatments S1 and S2 when compared with S0 in the June–October sample. However, they were not significantly different. The decomposition constant k (equation 3) did not show any significant difference among the three treatments at all durations. However, the slightly higher decomposition rate of litter was observed in earlier months in all three treatments. Our results showed that the decomposition process of the litter did not undergo significant change among the three treatments for all sampling periods. The concentrations and amounts of N in the litter (*I. cylindrica*) are shown in Fig. 1. The N loss from the litter was estimated by using the N concentration and weight difference for each sampling period. The linear decrease in the amount of N in the litter shows the quick release of N from the litter to soil within 2 months in all treatments. The total N released in June–October from *I. cylindrica* was highest in S2 (410 mg/m^2), and lowest in S0 (169 mg/m^2), but ANOVA did not show any significant difference. The estimation of N released by slashed weeds was significantly higher ($P<0.05$) in S2 (476 mg/m^2) compared to S0 (159 mg/m^2) (Table 4).

Soil physicochemical analysis

The soil physical and chemical changes in each treatment for the two sampling periods are shown in Table 5. Similar soil pH was observed in treatments S0 and S1 for both sampling periods. However, S2 showed a significant decrease in soil pH at the final sampling (November) when compared to treatments S0 and S1. Soil EC remained identical in all treatments throughout the experiment. Similar results were observed for the soil bulk density. The soil C concentration showed an increasing trend with the frequency of slashing; however, a significant difference was not observed between the treatments at final sampling. The soil N concentration significantly increased only in S2 treatment at the final sampling. Final soil N was higher than initial soil N in all the three treatments. Soybean was likely responsible for the increase in soil N concentration because of N fixation.

Nitrogen leaching

Nitrogen leaching was measured from June to November 2017. Nitrogen leaching did not show any significant difference among the three treatments, measured at two depths at 0-10 cm and 0-30 cm (Table 6).

Soil microbial biomass

Microbial biomass increased with the frequency of weed slashing. The total soil microbial biomass increased significantly on treatments S1(71.59 nmol/g) and S2 (68.66 nmol/g) compared to S0 (38.46 nmol/g) ($P<0.05$) at the final soil sampling. Gram-positive, gram-negative, and fungi did not show any significant difference among the three treatments within the initial and final stages. But a significant increase in gram-positive and gram-negative bacteria was observed in treatments S1 and S2 in November at the final stage when compared to the initial stage (Fig. 3). An increasing trend of fungal biomass was observed, but there were no significant differences over time among the treatments. PERMANOVA showed a significant change in soil microbial community structure among the three treatments only during the final stage ($P<0.05$) (Fig. 4).

Discussion

N release from weeds

Slashing weeds and allowing them to decompose *in situ* means that nutrients contained in weeds can be transferred to the soil and/or crops growing in the same location. Studies have demonstrated that the weeds consume a high amount of N from the soil (Hans and Johnson 2002), suggesting that weeds were good competitors against crops for nutrients and could be a good source of N. However, nutrient uptake, and competitiveness depends on the species trait of the weeds (Blackshaw and Brandt 2008). In our study, the estimation of N release from the mixture of all the weed species after slashing is a complex and challenging task because of different N content and decomposition rates among weed species. For example, *Bidens pilosa* had the highest C:N ratio, whereas *Chamaesyce maculate* had the lowest C:N ratio (Table 7). However, most of the weed species found in our experimental site had a lower C:N ratio compared to *I. cylindrica* (average: 47.6). Because we assumed that the N concentration and N released from weeds after slashing was similar to that of *I. cylindrica*, therefore our estimation of N release from weeds to soil was a conservative approximation. We also calculated the decomposition constant (k value), and it showed a slightly higher decomposition rate during the early months in all treatments. We found that the amounts of N released from the litter were faster during early-stage within two months (Fig 1), which agrees to general decomposition pattern as most of the liable elements are lost during early stages (Berg, 2000). These early litter decompositions may be due to the leaching of soluble compounds. (Heim and Frey, 2004). Simple soluble compounds are readily lost from litter through dissolution and leaching combined with the action of microorganisms. The leaching of water-soluble materials of the litter might be regulated by the same factors that regulate mass loss that is not physiologically modified by microorganisms until after leaving the litter (Djukic et al., 2018).These dissolved materials may be lost from litter to the soil. In such cases, the materials are lost from the litter but are retained in the soil ecosystem (Berg, 2000; Djukic et al., 2018; Heim and Frey, 2004).

In our study, the amount of N released by the litter and weeds were very small when compared with that of common rates of fertilizer application. But we showed that slashing weeds had a positive implication for N release to the soil. The difference in weed slash frequency resulted in a significant difference in weed mulch biomass and N release among the treatments. Weed acts as a nutrient accumulator during the early development stage of the crops. Furthermore, unlike compost and fertilizer preparations, weeds are immediately used as mulch, reducing the N loss during processing and transportation. The presence of weed roots also means that they can take up the N for their growth, and when aboveground biomass is slashed, dying roots become N source for crops. Hence, through our approximation, the weeds were able to release a substantial amount of N back to the soil and could be applied in agricultural fields as an N source. Therefore, we propose that the management of green weed slashing and immediate mulching can increase N release to the soil.

Soil microbial biomass

Slashing frequency affected both weed species and soil microbial group composition. Significant increases in total soil microbial biomass were observed in treatments S1 and S2 compared to S0. Weed slash and mulch did not affect the fungal biomass, whereas gram-positive and gram-negative bacterial biomass increased by 1.5-fold in treatments S1 and S2. Miura et al. (2013) found that weed mulching stimulated soil bacterial growth. Bacteria require more N per unit C accumulation than fungi (De Deyn et al. 2008). While other studies have shown that fungi were capable of colonizing and transferring N from freshly fallen litter to soil, bacteria were dependent on the movement of high-N concentration organic matter to their low-N concentration cells (Cotrufo et al. 2009). Likewise, plant diversity can modify the composition and function of microbial communities, which can influence the ecosystem processes (Choudhary et al. 2018). Eisenhauer et al. (2010) demonstrated that in a diversified grassland, the quality and the quantity of litter (available resources) strongly affected the microbial function in the soil. At harvest, all treatments had a maximum of approximately 10 weed species in this study. However, the quantity of weeds used as mulch in the treatments S1 and S2 was higher compared to S0. The root and total soil microbial biomass showed positive correlation between each other (Fig 5). The roots acts as organic matter in the soil and releases roots exudates and increases the soil microbial activity in rhizosphere (Grayston et al., 1996; Rasse et al., 2005). Thus, the amount of weed mulch and root biomass might have influenced the soil bacterial growth in treatments S1 and S2. The slashing treatments and soybean cultivation modified the weed community structure in this study. Here, the composition of the weed was not controlled; rather, the slashing was carried out to reduce the competition between the crops and the weeds. Thus, the slashing and mulching of the weeds assisted in the improvement of soil by increasing soil organic matter content and soil microbial activity.

Crop and weed management

In this study, slashing weed once (S1) was sufficient to reduce the competition between soybean and weeds, resulting in higher soybean yield compared to S0. Hence, the labor required to manage the weeds in an NTW farm in this particular condition could be reduced to one time slashing after seeding. Due to the presence of both weeds as well as crops during all periods, the plant diversity is relatively high in NTW practice. Other weed mulch practices like living mulch and cover crops have a comparatively low diversity of plants and are eventually slashed or mixed with the soil. Studies have shown that the diversity of plants and resource availability can enhance plant productivity, particularly in grasslands (Tilman et al. 2014). However, in agricultural lands, the impact of diversity is not well understood. Arai et al. (2014) showed that NTW increases aboveground productivity because of the presence of weeds, which act as a major N source. However, weeds competition for land, water, nutrients, and other resources still poses a threat to crop growth and hence should be addressed with proper management. The introduction of slash and mulch generally suppresses the growth of weeds by limiting the sunlight and reducing competition for resource accumulation. The appropriate management of weeds in CA systems remains unclear, however, our results showed that either slashing once or twice produced similar biomass of weeds at the end, which means that the competition between the crop and weeds in treatments S1 and S2 was similar.

Conclusion

Our study showed that weeds are an asset in the agriculture lands where it acts as N sink and can be utilized as a soil amendment. In our study, the amount of weed used as mulch was relatively low, resulting in a small release of N from the slashed weeds. Despite the limitation, I was able to control and utilize the weeds to increase the soybean yield in this study, without the use of any external resources. Increasing the slashing frequency showed an increase in soil organic matter, microbial activity, and weed N release. The root and microbial biomass showed positive correlation between each other. The highest soybean yield in our study was obtained after one time slashing of weeds. This finding means that I can reduce the labor required to manage the farm. Further research with alternative strategies such as increasing the amount of weed mulch or combined application of weed mulch and reduced fertilizer can be considered to increase the N recycling and crop yield.

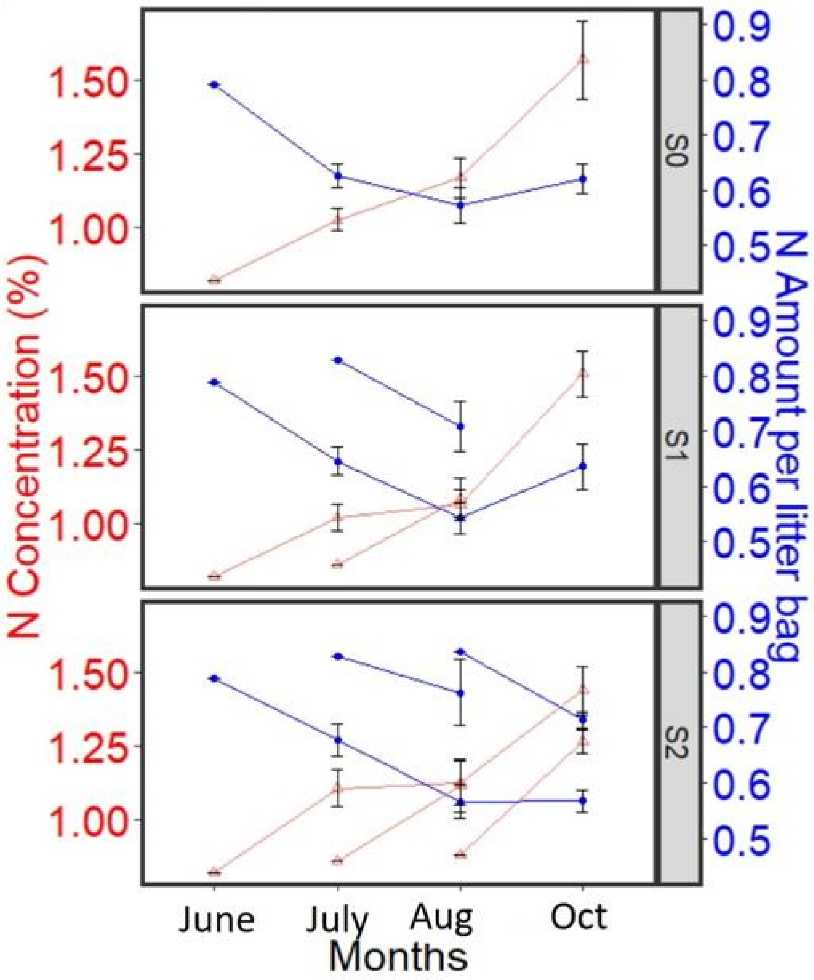

Figure 1. The N concentration (•) and N amount (Δ) during the decomposition process of *(Imperata cylindrica)* in the litterbag at three treatments. Only an initial weed slash was done in S0 treatment in June represented by the straight line. After the initial slash, the weed was slashed one time in the treatment S1 and S2 represented by a dashed line from July. In S2, weeds were slashed one more time, represented by second dashed lines from August. The N concentration increased while the N amount is decreased. The linear decrease in the amount of N in the litter shows the release of N from the litter to soil within 2 months in all treatments. However, no significant difference was observed in the N concentration as well as N amount among the three treatments during each month. Error bar = Standard deviation.

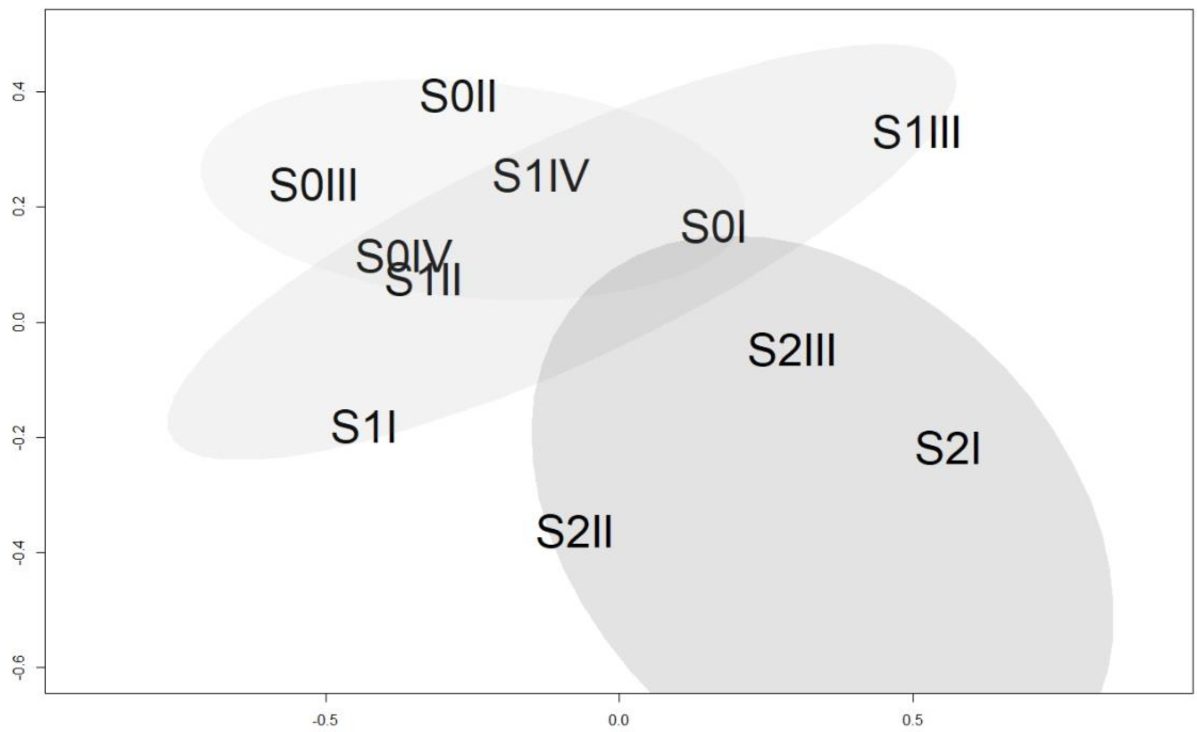

Figure 2. NMDS plot illustrating weed community structure among the three treatments during the harvest (October). PERMANOVA showed a significant difference at $p = 0.07$ among the treatments. S0, S1, and S2 represent the slashing frequency, i.e., slashing weeds zero, once and twice. The roman letter indicates the replication of the treatments.

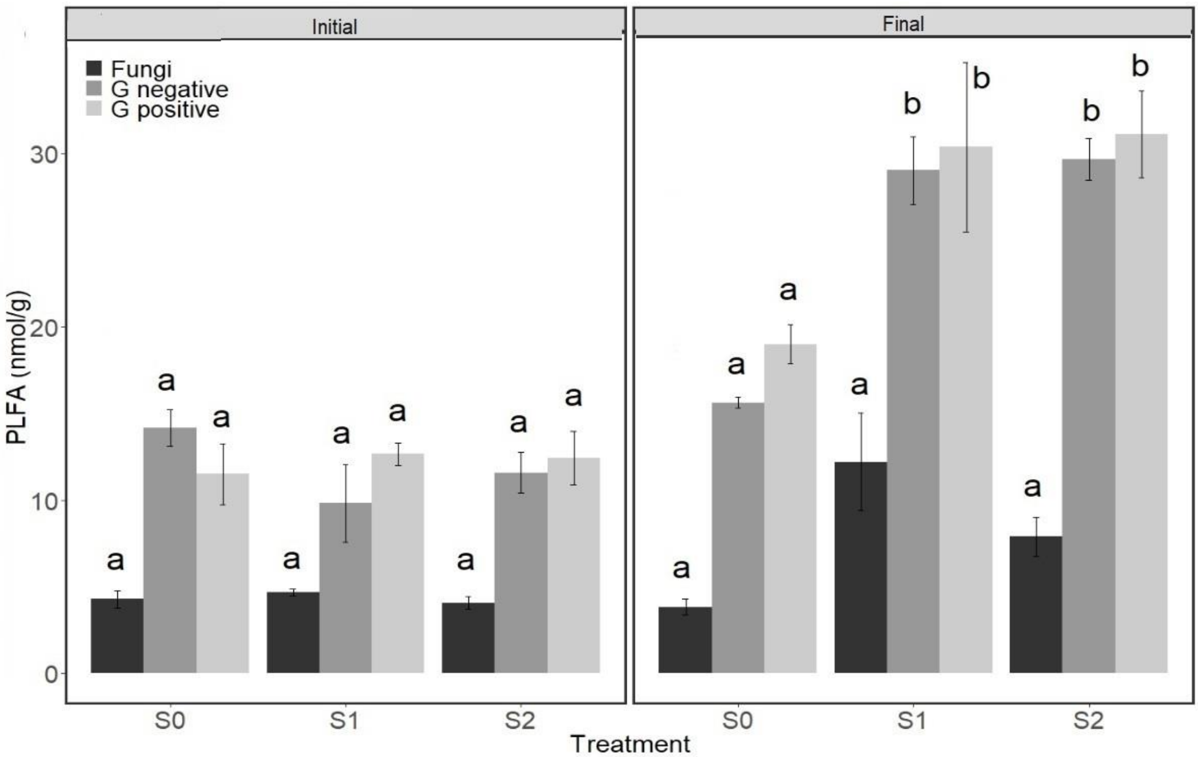

Figure 3. Comparison of soil microbial biomass at the initial and final soil samples on the three treatments. The initial soil sample was sampled in June 2017 and final in November 2017. Different alphabet represents a significant difference between the change of soil microbial biomass between the initial and final stages among the three treatments ($p < 0.05$, Repeated ANOVA). Gram-positive and gram-negative bacterial biomass on the final soil sample showed a significant increase in the treatments S1 and S2 compared to the initial soil sample. Error bar = Standard deviation.

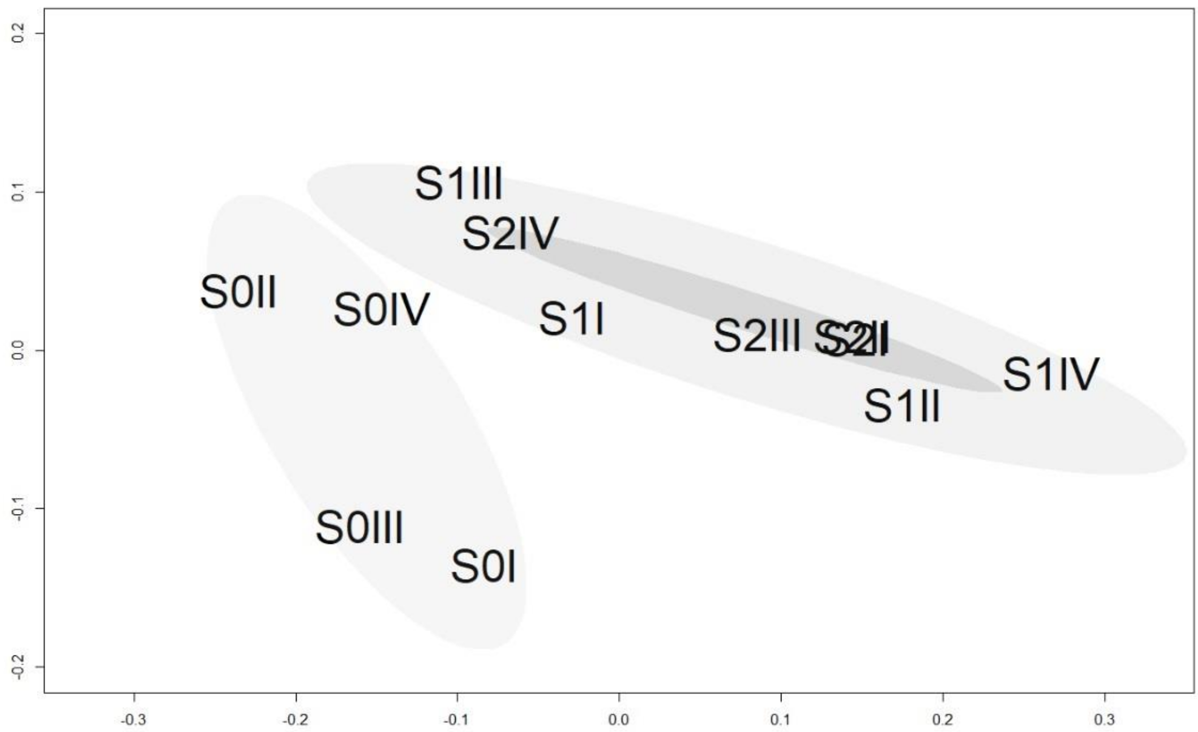

Figure 4. NMDS plot illustrating soil microbial community structure among the three treatments during the harvest (October). PERMANOVA showed a significant difference at $p < 0.05$ among the three treatments. S0, S1, and S2 represent the slashing frequency, i.e., slashing weeds zero, once and twice. The roman letter indicates the replication of the treatments.

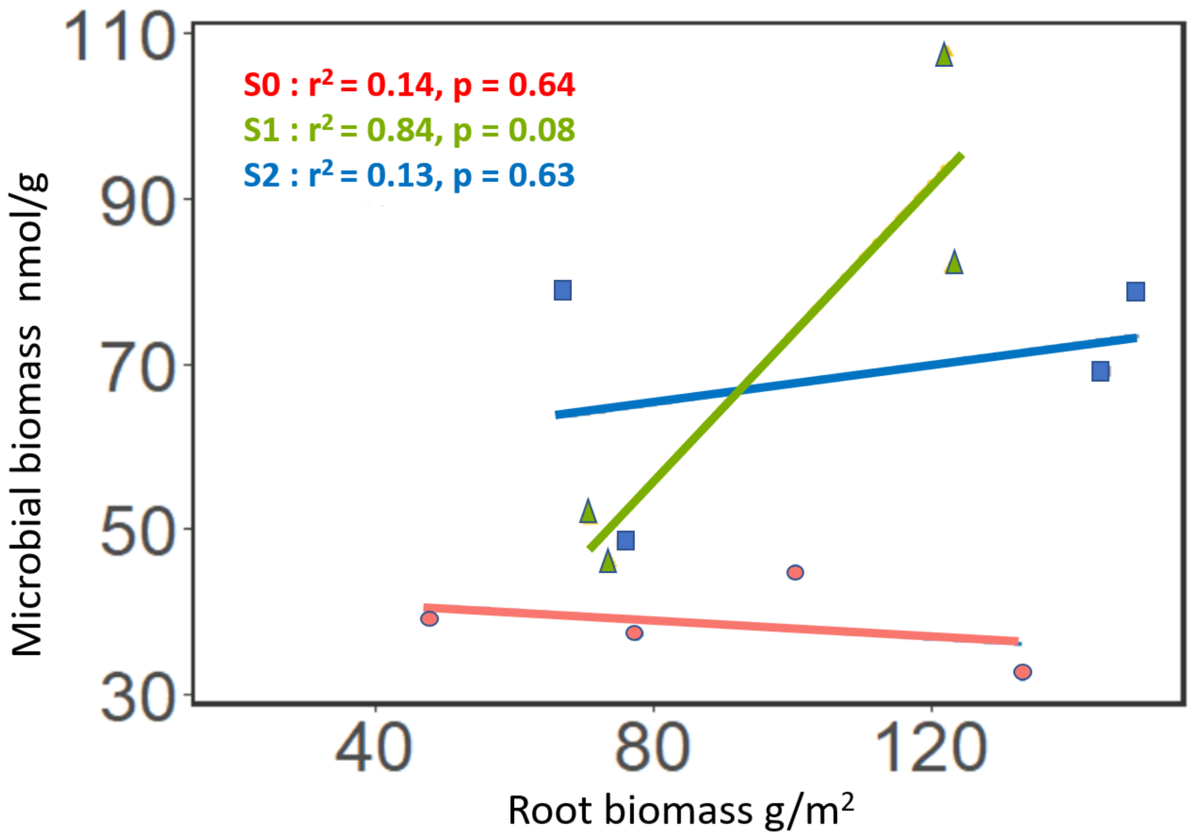

Figure 5. Correlation between Soil microbial biomass and root biomass in the three treatments. Treatment S1 and S2 showed higher soil microbial biomass and root biomass compared to the treatment S0. S1 showed significant correlation between soil microbial biomass and root biomass.

Table 1. Weed biomass and soybean grain yield (g/m^2) across three treatments.
Only the weeds slashed in June were added as mulch in S0, since I only did initial slash. I took the samples for other months to observe the change in weed composition. Similarly, weeds slashed in June and July were added as mulch for S1 and June to August for S2. Repeated measure ANOVA was performed to check the difference in weed biomass during different months in each treatment. Means with different letter represent significant difference among the treatments at each sampling (Tukey HSD test, $p<0.05$).

Treatments	Initial slash (June)				First slash (July)				Second slash (August)				At harvest (October)				Repeated measures	Soybean yield			
	Mean	±	S.D.		Mean	±	S.D.		Mean	±	S.D.		Mean	±	S.D.			Mean	±	S.D.	
Slashing zero (S0)	89.8	±	3.0	a	94.8	±	14.8	a	109.6	±	19.0	a	79.3	±	5.2	a	n.s	10.8	±	0.7	a
Slashing once (S1)	97.4	±	1.7	a	98.4	±	14.4	a	64.2	±	2.7	a	52.1	±	6.6	ab	n.s	21.4	±	1.0	b
Slashing twice (S2)	105.4	±	4.9	a	82.3	±	10.8	a	107.4	±	13.1	a	30.0	±	3.2	b	n.s	18.9	±	0.8	b

Table 2. Weed species composition in three different treatment during three different months.
X: present, -: absent

	Slashing zero (S0)			Slashing once (S1)			Slashing twice (S2)		
Weed species	July	August	October	July	August	October	July	August	October
Andropogon virginicus	X	X	X	X	X	X	X	X	X
Bidens frondosa	-	X	-	X	X	X	X	X	X
Bidens pilosa	X	X	X	X	X	X	X	X	X
Chamaesyce maculata	X	X	X	X	X	X	-	-	-
Chamaesyce nutans	-	X	-	-	X	-	X	X	-
Digitaria ciliaris	X	X	X	X	X	X	X	X	X
Erechites hieraciifolius	-	-	-	-	X	-	X	X	-
Equisetum arvense	X	X	X	-	-	-	-	-	X
Erigeron canadesnsis	-	-	X	X	-	-	X	-	-
Erigeron annuus	X	-	-	X	-	-	-	-	-
Hypochaeris radicata	-	X	X	X	X	X	-	X	X
Lactuca indica	X	X	-	X	X	-	X	X	-
Lespedeza cuneata	X	-	X	-	-	X	-	-	X
Lespedeza pilosa	X	X	X	X	X	X	X	X	X
Pueraria lobata	X	-	X	-	-	X	-	-	-
Setaria pallidefusca	X	X	X	X	-	X	X	X	X
Solidago altissima	-	-	-	-	-	-	X	X	X
Sonchus asper	-	-	-		X	X	-	X	X
Vicia hirsuta	-	-	-	-	X	-	-	X	-

Table 3. Parameters of litter bag content (*Imperata cylindrica*) in the three treatments at different durations. No significant difference was observed between the three treatments at each sampling time (ANOVA test).

			Treatments								
			Slashing zero (S0)			Slashing once (S1)			Slashing twice (S2)		
Duration	Months in the field	Litter parameters	Mean	±	S.D.	Mean	±	S.D.	Mean	±	S.D.
Initial (June)	0	C (%)	44.0	±	1.52	44.0	±	1.52	44.0	±	1.52
		N (%)	0.92	±	0.03	0.92	±	0.03	0.92		0.03
		C:N ratio	47.63	±	1.05	47.63	±	1.05	47.63	±	1.05
		Dry weight (g/litter bag)	7.40	±	0.21	7.40	±	0.21	7.40	±	0.21
June-July	1	C (%)	44.1	±	1.20	43.9	±	0.29	43.1	±	0.42
		N (%)	1.03	±	0.11	1.02	±	0.14	1.11	±	0.18
		C:N ratio	43.00	±	0.77	43.08	±	0.11	38.89	±	0.17
		Remaining dry weight (g/litter bag)	4.70	±	0.18	4.88	±	0.15	4.73	±	0.25
		Decomposition coefficient (k)	0.041	±	0.002	0.049	±	0.002	0.041	±	0.003
		Moisture content (%)	17.2	±	3.92	16.0	±	2.19	14.6	±	3.36
July-August	1	C (%)	46.9	±	0.69	48.4	±	1.02	46.7	±	1.65
		N (%)	1.09	±	0.20	1.08	±	0.20	1.12	±	0.27
		C:N ratio	42.90	±	0.34	44.69	±	0.58	41.85	±	0.98
		Remaining dry weight (g/litter bag)	5.20	±	0.27	5.06	±	0.23	5.27	±	0.19
		Decomposition coefficient (k)	0.043	±	0.003	0.044	±	0.003	0.042	±	0.003
		Moisture content (%)	29.2	±	3.59	24.8	±	5.31	26.6	±	2.44
June-August	2	C (%)	45.0	±	0.73	45.2	±	1.33	47.4	±	1.84
		N (%)	1.17	±	0.21	1.06	±	0.16	1.13	±	0.22
		C:N ratio	38.52	±	0.37	42.52	±	0.82	42.10	±	1.14
		Remaining dry weight (g/litter bag)	3.78	±	0.36	3.93	±	0.15	3.90	±	0.36
		Decomposition coefficient (k)	0.043	±	0.004	0.041	±	0.002	0.041	±	0.004
		Moisture content (%)	34.4	±	3.49	36.8	±	2.18	29.3	±	2.19
August-October	2	C (%)	44.8	±	0.67	44.2	±	0.83	43.9	±	1.24
		N (%)	1.11	±	0.13	1.18	±	0.13	1.27	±	0.12
		C:N ratio	40.23	±	0.38	37.52	±	0.50	34.59	±	0.79

		Remaining dry weight (g/litter bag)	4.13	±	0.15	4.43	±	0.32	4.35	±	0.24
		Decomposition coefficient (k)	0.048	±	0.002	0.049	±	0.002	0.047	±	0.002
		Moisture content (%)	29.6	±	5.14	38.1	±	7.13	22.6	±	2.35
June-October	4	C (%)	42.7	±	1.16	41.8	±	1.77	44.0	±	1.53
		N (%)	1.57	±	0.40	1.50	±	0.23	1.44	±	0.24
		C:N ratio	27.21	±	0.54	27.83	±	1.09	30.61	±	0.91
		Remaining dry weight (g/litter bag)	3.11	±	0.33	3.23	±	0.22	3.07	±	0.38
		Decomposition coefficient (k)	0.036	±	0.004	0.038	±	0.003	0.035	±	0.005
		Moisture content (%)	61.2	±	20.1	95.6	±	13.2	86.3	±	10.0

Table 4. Nitrogen (mg/m^2) released from the litterbag and the slashed weeds in each treatment (n=4). The weeds were slashed before seeding in June initial slash in all treatments and N released was measured from June to October. The first slash was done in July and the N release was again measured from July to August, and similarly, for the second slash, N release was measured from August to October. Litter bag containing *I. cylindrica* was placed to measure the N release. In S1 and S2 the N release during August-October from weed slashed in July was not measured. Hence, N release in S1 and S2 will be an underestimate. (-) shows that no slashing was performed.

	Imperata N release																		
	Initial slash									First slash			Second slash						
Treatment	June-July			July-August			August-October			July-August			August-October			Sum			Tukey HSD
	Mean	±	S.D.	Mean	±	S.D.	Mean	±	S.D.	Mean	±	S.D.	Mean	±	S.D.	Mean	±	S.D.	
Slashing zero (S0)	163.6	±	21.7	54.4	±	36.5	-49	±	36.2		-			-		168.9	±	8.5	a
Slashing once (S1)	144.1	±	24.4	103	±	49.6	-65	±	34.8	120	±	46		-		303.0	±	11.4	a
Slashing twice (S2)	112.1	±	29.3	112	±	52.6	-1.7	±	23.9	65.2	±	60	122	±	14.3	409.8	±	19.4	a
	Weed N release																		
	Initial slash									First slash			Second slash						
	June-July			July-August			August-October			July-August			August-October			Sum			
	Mean	±	S.D.	Mean	±	S.D.	Mean	±	S.D.	Mean	±	S.D.	Mean	±	S.D.	Mean	±	S.D.	
Slashing zero (S0)	148.5	±	20.1	44.8	±	31.63	-34	±	31.9		-			-		159.7	±	6.7	a
Slashing once (S1)	151.4	±	28.4	93.3	±	45.1	-59	±	42.6	124	±	70		-		309.6	±	17.2	ab
Slashing twice (S2)	119.9	±	35.0	136	±	59.54	-5.3	±	27.3	104	±	61	122	±	15.9	476.1	±	19.8	b

Table 5. Comparison of soil physical and chemical parameters among the three treatments at two different sampling times. Means with the different letters in the initial and final sampling periods are significantly different among the treatments (Tukey-HSD, P<0.05).

	pH				EC (mS/m)				Bulk density (g/m^3)				Carbon (%)				Nitrogen (%)			
Initial	Mean	±	S.D.		Mean	±	S.D.		Mean	±	S.D.		Mean	±	S.D.		Mean	±	S.D.	
Slashing zero (S0)	6.19	±	0.11	a	6.83	±	2.55	a	0.62	±	0.02	a	4.37	±	0.2	a	0.31	±	0.02	a
Slashing once (S1)	6.19	±	0	a	6.54	±	0.52	a	0.64	±	0.03	a	4.42	±	0.4	a	0.32	±	0.04	a
Slashing twice (S2)	6.26	±	0.1	a	5.07	±	1.15	a	0.65	±	0.04	a	4.20	±	0.03	a	0.28	±	0.02	a
Final	Mean	±	S.D.		Mean	±	S.D.		Mean	±	S.D.		Mean	±	S.D.		Mean	±	S.D.	
Slashing zero (S0)	6.21	±	0.06	a	6.61	±	3.04	a	0.41	±	0.02	a	5.25	±	0.01	a	0.43	±	0.42	a
Slashing once (S1)	6.18	±	0.2	a	6.28	±	2.00	a	0.40	±	0.04	a	5.39	±	0.07	a	0.45	±	0.54	ab
Slashing twice (S2)	6.05	±	0.10	b	7.94	±	2.73	a	0.42	±	0.02	a	5.83	±	0.1	a	0.49	±	0.36	b

Table 6. Ammonium and nitrate (g/m^2) in the three treatments at two depths (10 cm and 30 cm). No significant change was observed between the treatments both in ammonium and nitrate amount at two depths.

Treatment	Depth (cm)	Ammonium			Nitrate		
		Mean	±	S. D.	Mean	±	S. D.
Slashing zero (S0)	10	0.09	±	0.03	0.09	±	0.04
	30	0.04	±	0.01	0.08	±	0.04
Slashing once (S1)	10	0.05	±	0.01	0.1	±	0.05
	30	0.05	±	0.01	0.04	±	0.01
Slashing twice (S2)	10	0.05	±	0.01	0.06	±	0.02
	30	0.07	±	0.01	0.06	±	0.01

Table 7. C:N ratio of weed species found in July.
Average C:N of *I. cylindrica* in July was 51.5

	Slashing zero (S0)	Slashing once (S1)	Slashing twice (S2)
Weed species	C: N	C: N	C: N
Andropogon virginicus	32.2	35.3	29.7
Bidens frondosa	-	18.9	19.2
Bidens pilosa	72.4	47.9	34.5
Chamaesyce maculata	11.2	15.9	-
Digitaria cillaris	46.1	38.7	28.6
Equisetum arvense	24.3	-	-
Erigeron annuus	17.9	20.5	-
Erechites hieracifolius	-	-	17.9
Hypochareis radicata	24.2	21.4	18.3
Lespedeza cuneata	15.8	-	-
Lespedeza pilosa	25.7	24.8	26.5
Pueraria lobata	14.3	20.0	-
Setaria pallidefusca	54.0	29.8	28.2
Solidago altissima	-	-	41.4

Chapter 4

Estimation of nitrogen budget of no-tillage with weed management system

Introduction

The population of the world is expected to reach 9.1 billion by 2050 (FAO, 2009), and more food production is required to support the increasing population. However, both agricultural lands and natural resources such as water, NPK based fertilizers are limited (Godfray et al., 2012). Increasing agricultural food production worldwide over the past four decades has been associated with a 7-fold increase in the use of anthropogenic sources of reactive nitrogen (Galloway et al., 2008). Reactive nitrogen (Nr) is defined a diverse pool of nitrogenous compounds which are biologically, photochemically and radiatively active forms, which includes organic compounds (e.g., urea, amines), mineral N forms, such as NO_3^- and NH_4^+ as well as gases that are chemically active in the atmosphere (NOx, NH_3, N_2O). The increase in the application of Nr has continued to deteriorate the diversity and functioning of soil micro-organisms, animal, and plant ecosystems. The availability of inexpensive nitrogen fertilizer all over the world made farmers apply excessive amounts of nitrogen in their farmland (Cassman et al., 2002). Therefore, in recent years, a significant gap has occurred between the quantity of nitrogen fertilizer added to the agriculture land and the amount of nitrogen actually used by the crops (Tilman et al., 2002). The unused nitrogen is susceptible to leaching, runoff, and volatilization, which have a severe impact on the environment like eutrophication and global warming. In a review paper (Raun and Johnson, 1999) have calculated that about 67% of applied fertilizer represents a $15.9 billion annual loss of N fertilizer and that even a 1% increase of nitrogen use efficiency (NUE) would result in global savings of $234 million. These authors suggest that the best hope for reducing the need for fertilizer N lies in finding more efficient ways to deliver fertilizer. It is, therefore, of major importance to identify the critical steps controlling plant N use efficiency. Moll *et al.* (1982)defined NUE as being the yield of grain per unit of available N in the soil (including the residual N present in the soil and the fertilizer). However, there are many indices for calculating

NUE. But the overall objective of nutrient use efficiency is to increase the overall performance of cropping systems by providing economically optimum nourishment to the crop while minimizing nutrient losses from the field and supporting agricultural system sustainability through contributions to soil fertility or other soil quality components. In order to solve the agriculture-derived nitrogen problem, it is necessary to improve the NUE of crops in agricultural land and reduce nitrogen fertilizer usage (Canfield et al., 2010; Tilman et al., 2002)

Nitrogen use efficiency is extensively studied, and there are a plethora of studies and methods to optimize NUE depending on the crop cultivar, land management, and fertilizer input methods. Practices including prescriptive fertilizer management, using enhanced fertilizer, GMO crops that are efficient on N utilization are some of the examples (Giller et al., 2004; Liu et al., 2015; Sharma and Bali, 2018; Vitousek et al., 1997). Ecological methods like legume plantation, cover crops, no-tillage, conservation agriculture have also been widely researched (Choi et al., 2016; Qin et al., 2015; Tai-wen et al., 2018). A global study on cereal NUE showed that compared to 1999, United States improved its NUE by 10% from 2002 to 2015 due to improvement in cultivars and precision crop management (Omara et al., 2019). In most cases, improvement in NUE requires an investment of resources on external factors like crop cultivars, enhanced fertilizer or usage of cover crops/mulch, herbicides, and pesticides for practice like no-tillage.

No-tillage with weed (NTW) is one of the conservation agricultures practices where naturally available weeds are grown together with the crops. The above-ground biomass of the weeds is slashed and used as a mulching resource on the soil surface. NTW system is independent of external resources and utilizes locally available resources to conserve the soil ecosystem and maintain crop productivity. Since no extra maintenance is required to grow the weeds, they can be used as an N source for the soil, especially in small scale resource-poor farmers. NTW practice has high plant diversity, which has the potential to increase the biodiversity of the soil, reduce N leaching and increase soil organic matter (Arai et al., 2014; Kaneko, 2014; Miura et al., 2013; Yagioka et al., 2015). The present study was conducted to determine if both weeds and plants could be sustained in the same field during the introduction of no-tillage with weed management and whether they co-exist or compete against each other for nutrition. I hypothesize that the NTW system will have more efficient nitrogen usage due to the presence of weeds as well as crops.

Materials and methods

Site description

This study was conducted at Yokohama National University (139° 35' 20"E, 35° 28' 20"N, Elevation 58 m) in Yokohama, Japan. The soil was black volcanic soil (Andisol USDA Soil Taxonomy). The study site was grassland for 30 years before the research began in 2010 (Tohma et al., 1994). Winter wheat was planted from November 2014 to June 2015, during which time the annual precipitation and average temperature were 1140 mm and 13.8°C, respectively. The site consisted of four blocks, each containing four plots (total 16 plots) of 2.5 m × 2.5 m. Each plot was further divided into three rows of approximately 0.9 m × 2.5 m. All blocks were assigned one of the following four treatments, with tillage and fertilizer being the primary factors: tillage with fertilizer (TF), tillage without fertilizer (TC), no-tillage with fertilizer (NF) and no-tillage without fertilizer (NC). The tillage plot was tilled with a small tractor. In the no-tillage plot, weed management was accomplished by cutting the weeds on the surface without disturbing the roots. The weeds were used as mulch in the NTW plot. Fertilizer with N: P: K 8:8:8 was applied in the NF and TF plots (570 g per plot). The amount of nitrogen input was 50 kg/ha. Winter wheat (var. Yumechikara) was planted on November 6, 2014 and harvested on June 1, 2015.

Soil analysis

Soil samples were collected from three different depths (0–5 cm, 5–10 cm, and 15–20 cm) using a 100 cm^3 core. The weight of soil samples was measured, after which they were oven-dried at 105°C for 24 hours. These samples were used to calculate soil physical and chemical properties such as pH, EC (electrical conductivity), bulk density, water content, and C and N concentration.

The pH and EC in soil and water solutions (1:5) using a pH/EC meter (pH/COND METER HORIBA, Kyoto, Japan). Total carbon and total nitrogen content were analyzed using a Macro Coder JM1000CN (J-Science Lab Co., Ltd., Kyoto, Japan).

Plants analysis

Wheat was harvested and sorted according to the plot, after which the grains and straw were separated and dried in an oven dryer at 40°C for 2 days. The sample was then ground and used for the analysis of C and N. Weeds were sampled from a 100 cm × 30 cm area using a measuring scale. Weed species were identified and labeled in accordance with the plots. Sampled weeds were oven-dried at 45°C for 3 days, after which the dry weight was measured, and the samples were ground with a food processor. The sample was then analyzed for C, and N. Nitrogen flux in plants was calculated by the total nitrogen in plants divided by the total N pool in the soil.

Root analysis

The soil was collected from 0–5 cm using a 400 cm^3 core. A sieve (0.2 mm opening) was used to separate the soil and the weed roots. The roots were then washed and air-dried, after which the root biomass was measured.

Soil fauna analysis

Pitfall traps were set in each plot to collect the soil macrofauna. An empty plastic cup with an 8 cm diameter was used as a trap. The trap was placed for 24 hours, after which all animals were collected. Following extraction, the trap was reinstalled, and this process was continued for 3 days. The collected samples were stored in the freezer until analysis. Samples were freeze-dried and then identified under a VHX 1000 digital microscope (KEYENCE, Osaka, Japan), after which the species number and the total number of faunas were recorded. Earthworms were collected using a quadrat. The soil was collected from a 50 cm x 50 cm quadrat, and earthworms were hand sorted. The samples were cleaned, and their biomass was measured.

Soil microbial biomass

Phospholipid fatty acid (PLFA) analysis was used to determine the microbial biomass in the soil. Briefly, 0–5 cm of soil from a 50 cm × 50 cm quadrat was used for extraction for PLFA analysis. The sample was then stored in the freezer until it was subjected to analysis. PLFAs were extracted from 1 g finely ground freeze-dried samples using a procedure based on Frostegård et al. (2010). Lipids were extracted with one-phase chloroform-methanol-phosphate buffer, after which the PLFA fraction was separated using silicic acid columns (BOND ELUT LRC-SI; Varian, Palo Alto, CA, USA) before trans-esterification with mild alkali and a final uptake in dichloromethane. Methyl non- adecanoate (19:0) was added to each sample as an internal standard. The fatty acid methyl esters were separated by gas chromatography using a Sherlock Microbial Identification System (MIDI, Newark, DE, USA). The fatty acids 15:0iso, 15:0anteiso, 16:0iso, 17:0iso and 17:0 anteiso were used to estimate gram-positive bacterial biomass, 16:1ω7c,16:1ω5c, 17:0cyclo, 18:1ω7c and 19:0cycloω8c were used to estimate gram-negative bacterial biomass, 16:1ω5c was used to determine arbuscular mycorrhiza and 18:2ω6,9 was used to determine fungal biomass (Frostegård et al., 2010; Gómez-brandón & Domínguez, 2010; Ringelberg et al.,1997).

Nitrogen leaching

Ion exchange resin (IER, mixed anion, and cation, Amberlite MB-1, ORGANO, Tokyo, Japan) was assembled in a circular disk made from PVC pipe (outer diameter 6.0 cm, inner diameter 5.1 cm, height 1.0 cm) with one side covered in nylon mesh. 25 ml (12 g dry weight) IER was filled in the disk and inserted in a small bag made of a stocking. The prepared resin bag was then buried at a depth of 25 cm depth in each plot. The bags were inserted from the periphery of the plot, so the soil structure on the plot was not disturbed. A string was then attached to the bag to determine the location. The bags were set up at the beginning of the experiment and retrieved after harvest. Upon recovery, the IER was dried for over 24 hours at 40°C, after which the dry weight was measured. The sample was then stored in the freezer at -10°C until it was subjected to inorganic nitrogen analysis. For measurement of inorganic nitrogen, 2.5 mg dry resin was measured in a flask, after which 25 ml 2 N KCL was added and shaken for 1 hour at 170 rpm. The solution sample was then filtered, and ammonium nitrogen (NH_4-N) and nitrate-nitrogen (NO_3-N) were extracted with stock solutions (same concentration as 2N KCL). The analysis was performed using an autoanalyzer (Future, Alliance Instruments, Frépillon, France).

Nitrogen use efficiency (NUE)

There are different kinds of NUE indices that are used to evaluate the efficiency of applied N. Three indices were used to evaluate NUE in my experiment, i.e., Partial factor productivity (PFP), Agronomic Efficiency (AE_N) and Crop recovery efficiency (RE_N). These three indices were selected because PFP is most common and shows the use efficiency of applied N resource, AE_N changes due to crop and soil management practice, which is the purpose of the NTW system, while RE_N is affected by the N application amount and N uptake by plants (Dobermann, 2005). Partial factor productivity (PFP) was calculated by using following equation.

$$PFP_N = (Y_N/F_N) \quad (1)$$

Where,

- Y_N = Crop yield with applied N (kg ha^{-1}) (TF and NF only)
- F_N = Amount of (fertilizer) N applied (kg ha^{-1})

Agronomic Efficiency was calculated by using the following equation.

$$AE_N = (Y_N - Y_0)/F_N \quad (2)$$

Where,

- Y_N = Crop yield with applied N (kg ha^{-1}) (TF and NF)
- Y_0 = Crop yield in a control treatment with no N (kg ha^{-1}) (TC and NF)
- F_N = Amount of (fertilizer) N applied (kg ha^{-1})

I evaluated AE_N for no-tillage with weed (NTW) and tillage (Till) treatments using the following equations.

$$AE_{Till} = (Y_{TF} - Y_{TC})/F_{TF} \quad (3)$$

$$AE_{NTW} = (Y_{NF} - Y_{NC})/F_{NF} \quad (4)$$

Similarly, crop recovery efficiency was calculated by using following equation

$$RE_N = (U_N - U_0)/F_N \quad (4)$$

Where,

- U_N = N uptake by the plant in the fertilized plot (kg ha^{-1}) (TF and NF)
- U_0 = N uptake by plant with no N (kg ha^{-1}) (TC and NC)
- F_N = Amount of (fertilizer) N applied (kg ha^{-1})

I evaluated RE_N for no-tillage with weed (NTW) and tillage (Till) treatments using the following equations.

$$RE_{Till} = (U_{TF} - U_{TC})/F_{TF} \quad (5)$$

$$RE_{NTW} = (U_{NF} - U_{NC})/F_{NF} \quad (6)$$

Statistical analysis

Analysis of variance (ANOVA) was used to evaluate the effects of tillage and no-tillage on fertilization with pH, EC, carbon, and nitrogen content in the soil, wheat, and weeds, with blocks as a random factor. When significant differences were observed upon ANOVA ($p<0.05$), Tukey's-HSD was performed to compare different parameters among treatments. All data were analyzed using the statistical program R (version 3.2.3; Core Team, 2015).

Results

Soil

Both tillage and fertilization factors had significant effects on soil pH ($P<0.05$) (Table 1). The top (0–5 cm) layer of soil in NF showed a significantly lower pH than the other three treatments (Tukey's-test, $P<0.05$). In the 5–10 cm layer, pH was significantly lower in the fertilized plots (NF and TF) than the unfertilized plots (NC and TC). No differences were observed in the lowest layer (15–20 cm) of the soil. On the top layer of soil, significantly lower EC was observed in the tillage plots than the non-tillage plots ($P<0.05$). However, no difference was found in the other two layers. The total carbon and nitrogen pools were significantly higher in the no-tillage plots than the tillage plots in the top layer (0–5 cm). Fertilization had no significant effect on the total carbon and nitrogen concentrations in the soil. Moreover, no differences in any parameters were observed in the lower two layers (5–10 cm and 15–20 cm) of the soil.

Wheat yield

The highest yield of grain was 2165 kg/ha and 1656 kg/ha, and these were observed in the no-tillage fertilized (NF) and tillage fertilized (TF) plots, respectively (Fig. 1). Conversely, the no-tillage with no fertilizer (NC) and tillage with no fertilizer (TC) plots showed significantly less yield, i.e., 557 kg/ha and 525 kg/ha, respectively). No significant difference was observed between the tillage treatments (TF and TC) and no-tillage treatments (NF and NC), which implies that the no-tillage system has the potential to produce a similar amount of yield as the tillage system.

Roots biomass

Significantly higher root biomass was observed in the no-tillage plot (NF) than the tillage plots (TC and TF) ($P<0.05$). The highest biomass was observed in the NF plot (0.33 g/m^2), whereas the lowest biomass was observed in the TC plot (0.03 g/m^2) (Fig 2).

Soil microbial biomass (PLFA)

The results showed similar total soil microbial biomass in treatments NC (74.31 nmol/g) and NF (54.20 nmol/g). However, NC had significantly higher soil microbial biomass compared to tillage treatments TC (41.65 nmol/g) and TF (43.50 nmol/g) ($P<0.05$). Tukey-HSD test did not show any significant difference between gram-positive and negative bacteria as well as fungal biomass among the treatments (Fig 3).

Soil macrofauna

The total biomass in the no-tillage (NF) plot was found to be highest among plots, but the highest number of species was found in the tillage plot (TF) ($P<0.05$). The dominant species in NF was Isopoda (*Armadillidium vulgare*), at 0.42 g/plot, while there was a total of nine taxa of macrofauna in the TF plot. Isopoda, Hymenoptera, and Coleoptera were the most common animals in all treatments (Table 2). Similar results were obtained in earthworm biomass. Earthworms biomass in NF (10.9 g/m^2) was highest compared to NC (0.55 g/m^2), TF (0.003 g/m^2) and TC (0.005 g/m^2).

Nitrogen content in plants

The amounts of nitrogen in the wheat in the fertilized plots (TF and NF) and unfertilized plots (TC and NC) did not differ significantly. Weeds in no-tillage plots (NC and NF) had high nitrogen uptake compared to tillage plots (TC and TF) (Table 3).

Nitrogen flux

The total nitrogen uptake in plants from soil was very low (e.g., 3.89% of total soil N uptake in NC plots). As shown in Fig. 4, the total nitrogen uptake by the plants (both crops and weeds) in the treatments NC, NF, and TF were significantly higher than in the TC. Similar results were observed when weeds N uptake was compared among the four treatments. However, wheat N uptake did not show any significant difference among the four treatments.

Nitrogen leaching

Tillage and no-tillage did not show any significant difference in both ammonium and nitrate amounts. However, fertilized plots showed a significant increase in nitrate leaching, while ammonium was not affected significantly. The total inorganic nitrogen (ammonium + nitrate amount) showed a significant difference where the fertilizer was applied (Table 4).

Nitrogen use efficiency (NUE)

The PFP_N, AE_N and RE_N were significantly higher in no-tillage treatment compared to tillage treatment. PFP for no-tillage and tillage was 44 kg kg^{-1} and 33 kg kg^{-1} respectively. The AE_N for no-tillage was 32.17 kg $kg^{-1,}$ while a lower value of 22.62 kg kg^{-1} was observed for tillage treatment. Similarly, RE_N for no-tillage was 0.55 kg $kg^{-1,}$ while for tillage, it was 0.35 kg kg^{-1}.

Discussion

The weeds in no-tillage did not reduce wheat growth under fertilization. The wheat production in no-tillage plots with and without fertilizer (NF and NC) was similar to that of tillage plots with and without fertilizer (TF and TC), respectively. The average wheat yield for Kanagawa Prefecture in 2018 was 2850 kg/ha, which is slightly higher than our results (2165 kg/ha). However, the standard amount of fertilizer used to produce wheat was 100 kg/ha (Kanagawa prefecture fertilizer application standards, 2018), which is double the amount that was used in this study. Here, NTW was able to produce a similar yield with half the amount of fertilizer standardized for Kanagawa prefecture. The reduction in fertilizer requirement for the crop growth in NTW may be due to higher total N content on the soil pool and better nitrogen use efficiency compared to tillage treatment. The nitrogen released by the slashed weed might also have contributed to the soil N pool that was not evaluated in this experiment. The evaluation of the total nitrogen pool in the top layer of the soil revealed that the total nitrogen pool in the no-tillage treatments was higher than that in the tillage treatments (Fig 2). The higher soil nitrogen pool and plant nitrogen uptake indicate that nitrogen availability was higher in the no-tillage plots than the tillage plots. The amount of fertilizer in NF and TF was 5 g/m^2, which was 1% of the total soil pool. Moreover, the addition of the same amount of fertilizer in no-tillage (NF) and tillage (TF) resulted in the NF sustaining both plants and weeds. This suggests that both crops and weeds were able to efficiently utilize nitrogen in NF. The nitrogen use efficiency can be increased by reducing the N losses to the surrounding environment or increasing the N uptake by the plants. The main characteristic of NTW is to grow weeds together with the crops and slash and mulch the weeds as a source of N. This practice creates a closed system as the nutrients are taken up by weeds and is transferred back to the soil and vice-versa. Our results showed lower NUE in tillage treatment compare to NTW treatment, which included the weeds. No-tillage treatment showed 33%, 40% and 50% higher PFP_N, AE_N and RE_N compared to tillage treatment, respectively. This shows that crops in NTW treatment improve the N recovery from the applied N efficiently. The significant increase of NUE indices shows that NTW is more efficient in resource utilization compared to conventional practice. The results also showed similar crop yield in both no-tillage and tillage treatments, but higher N efficiency was achieved in a no-tillage system. Tilman et al. 2001 showed that plant diversity significantly increased both plant productivity as well as resource retention. The presence of weeds increases the above ground plant diversity and also increases the amount of root in the soil. The availability and physiological capacity of roots is one of the key mechanisms by which plants acquire the available nutrients (Bassirirad, 2000). Here, NTW showed the potential to increase the NUE by promoting root growth due to presence of weeds as well as crop (Fig 5).

Compared to tillage treatments, the management practice in no-tillage treatment consists of undisturbed landscape where weeds are a stable community. Weeds in no-tillage treatment may have competed with the crops leading to lower yield in NC. However, external fertilizer was supplied in NF treatment after 2 weeks of seeding. It is possible that the extra nitrogen input to the soil reduced competition between the crops and weeds relative to the unfertilized plot (NC). The amount of plant N uptake was similar in NC and NF which shows the potential for better crop production without fertilization. Hence, a better management technique to control the weeds can result in higher crop production in the NC plot as well. Although NC had a significantly lower yield compared to NF treatment, however the total plant uptake was similar.

Plant diversity has been found to impact ecosystem processes by adjusting the composition and function of microbial communities in the soil. Plant diversity, along with soil cover has been found to accommodate higher microbial respiration due to higher levels of plant diversity and availability of mulch materials (Choudhary et al., 2018; Miura et al., 2013). I observed higher root biomass in no-tillage treatment that can increase the microbial biomass and facilitate nutrient cycling and reduce N leaching (Katterer 2011, Bender and van der Heijden 2014). In our study, the total microbial biomass was significantly higher in no-tillage and no-fertilizer plot (NC). Generally, in long-term conventional agriculture studies, the addition of fertilizer facilitates the growth of microbial activity (Geisseler and Scow, 2014). However, in undisturbed lands, the application of fertilizer can reduce microbial activity. Studies have found that the addition of N can lead to changes in plant species composition and diversity, which in turn may affect the microbial community (Clark et al., 2007; Cleland and Harpole, 2010). Additionally, N input can decrease soil pH, leading to the mobilization of aluminum and the leaching of nutrient cations (Vitousek et al., 1997). I also found similar results as NF showed significantly lower pH compared to other treatments. Besides fertilization, studies have shown that there is an increase in microbial biomass when no-tillage is practiced because of changes in soil physical structure, moisture, temperature, and presence of roots exudates (Feng et al., 2003; Mathew et al., 2012; Miura et al., 2013), which can increase aboveground plant productivity across the ecosystems (Sackett et al., 2010). Tillage generally has a greater impact on larger soil organisms than smaller organisms (Robertson et al., 1994; Wardle et al., 1999). Hence, a lower number of organisms may be observed in disturbed land. In the present study, the no-tillage treatment showed significantly higher macrofauna biomass than the tillage plot (Table 3). Other pitfall trap studies have also shown increased predators and prey in no-tillage systems because of reduced mechanical disturbance to the soil (Marasas et al., 2001).

Conclusion

In this study, no-tillage with weed plots showed similar yields as tillage plots. High nitrogen availability and slashing and mulching of weeds reduced competition and promoted crops and weeds to grow together in the no-tillage plots. Weeds are generally considered to be detrimental in agricultural land, but in the NTW system, they acted as reusable resources. In the no-tillage system, significant increases in the root, macrofauna, and microbial biomass were observed, which may have resulted in the efficient use of nitrogen.

Conservation agriculture is an application of modern agricultural technologies to improve production while simultaneously protecting and enhancing the land resources on which production depends. Practices like NTW offer a new way of effectively and efficiently managing agricultural environments and natural resources. Future crop production will need to be more sustainable, as more biomass will need to be produced from less land with greater efficiency while minimizing the impact on the environment. NTW can play an important part in the future of agriculture and help overcome various agricultural difficulties associated with food production and resource management.

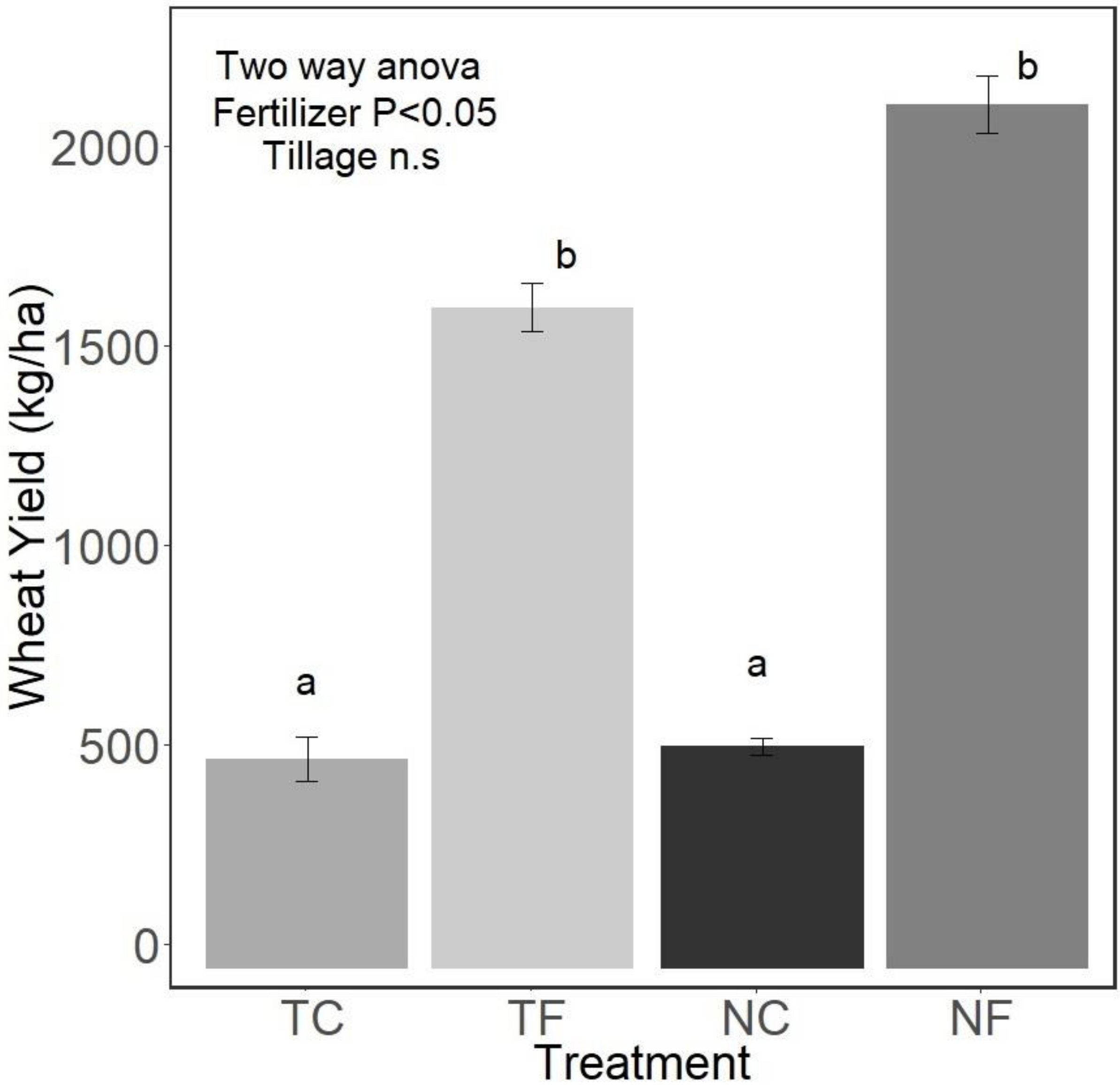

Figure 1. Wheat yield in across four treatment. Different alphabet represents a significant difference between the change of wheat yield among the treatments ($p < 0.05$, ANOVA). Tillage did not show any difference, but a significant increase in yield was observed with fertilization.

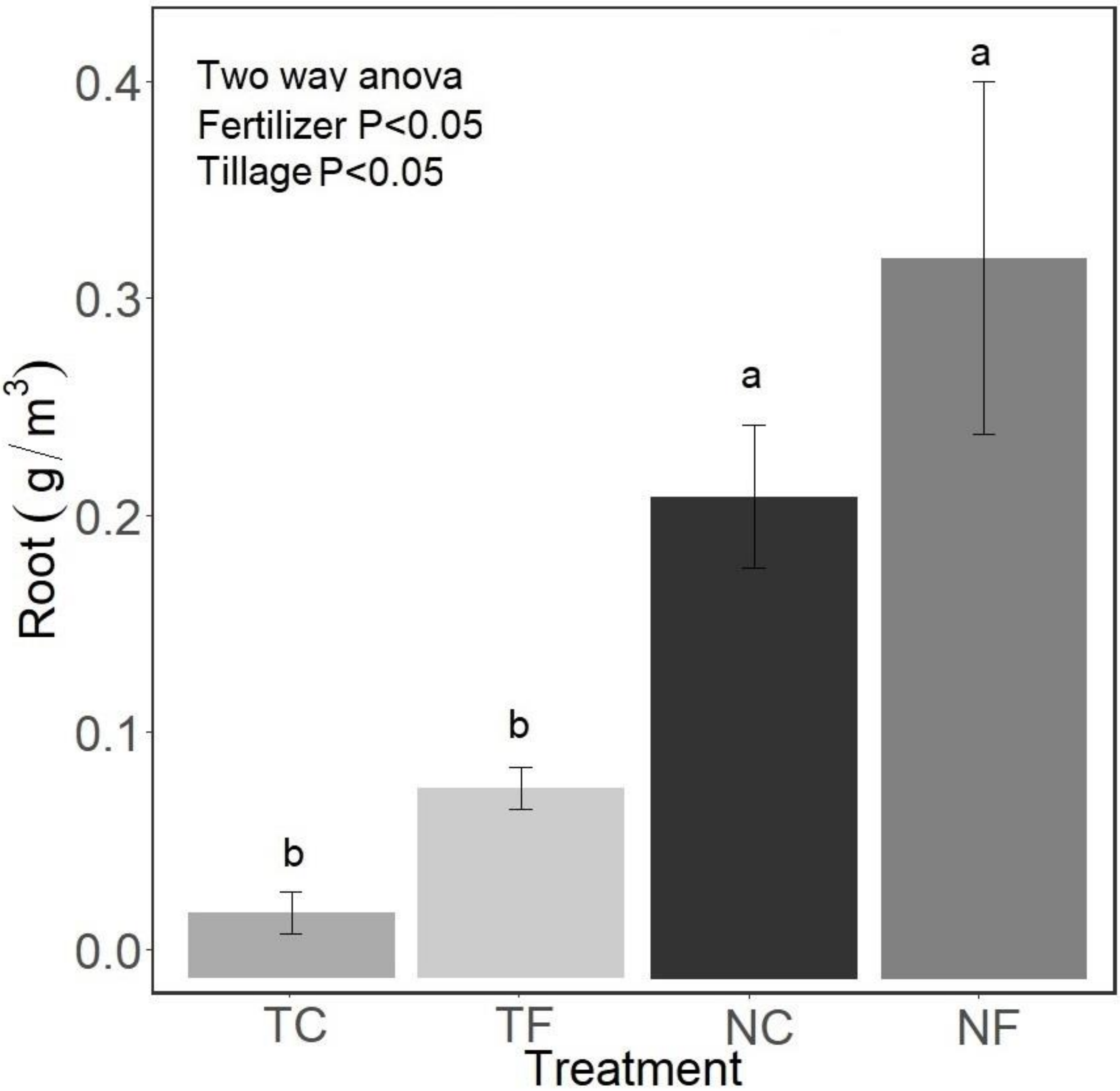

Figure 2. Root biomass on the four treatments. Different alphabet represents a significant difference between the change of root biomass among the treatments ($p < 0.05$, ANOVA). Tillage significantly reduced the root biomass.

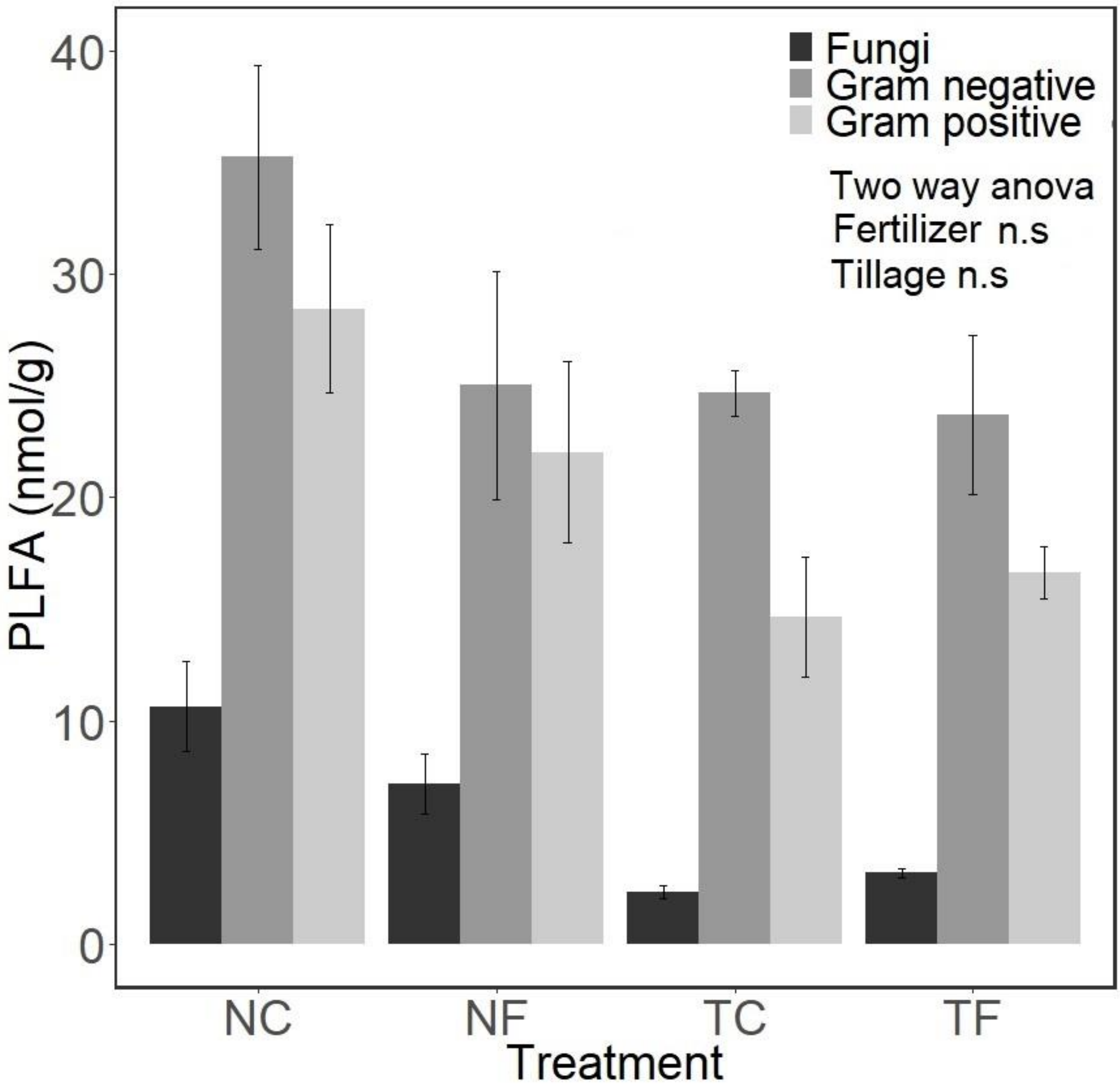

Figure 3. Fungi, gram-positive, and gram-negative bacteria in the four treatments. No significant difference was observed among the treatments. The total biomass (not shown) was significantly higher in NC treatment compared to TC and TF.

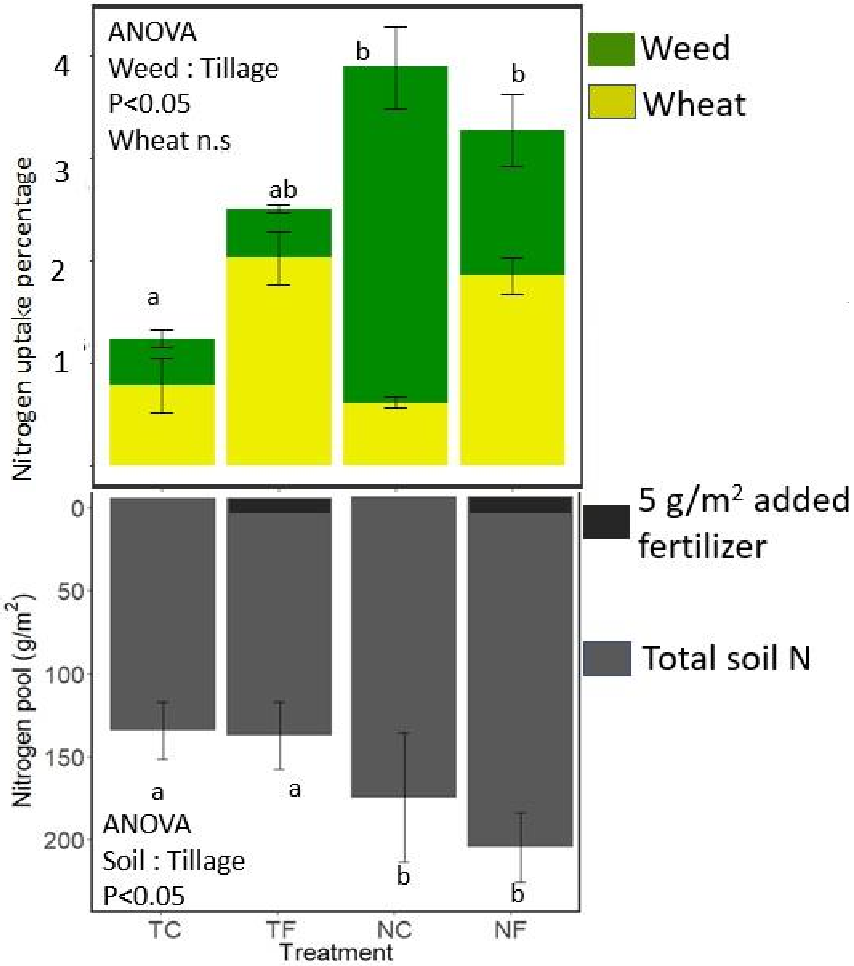

Figure 4. The total nitrogen pool in soil (below) and nitrogen uptake by weeds and wheat (above) from the soil. Tillage treatment significantly reduced the total nitrogen content on the soil. The total nitrogen uptake by weeds was significantly higher in NC and NF, while wheat did not show any significant difference. The total nitrogen uptake by both weed and wheat was significantly higher in TF, NF, and NC, where NC had the highest N uptake among the treatment.

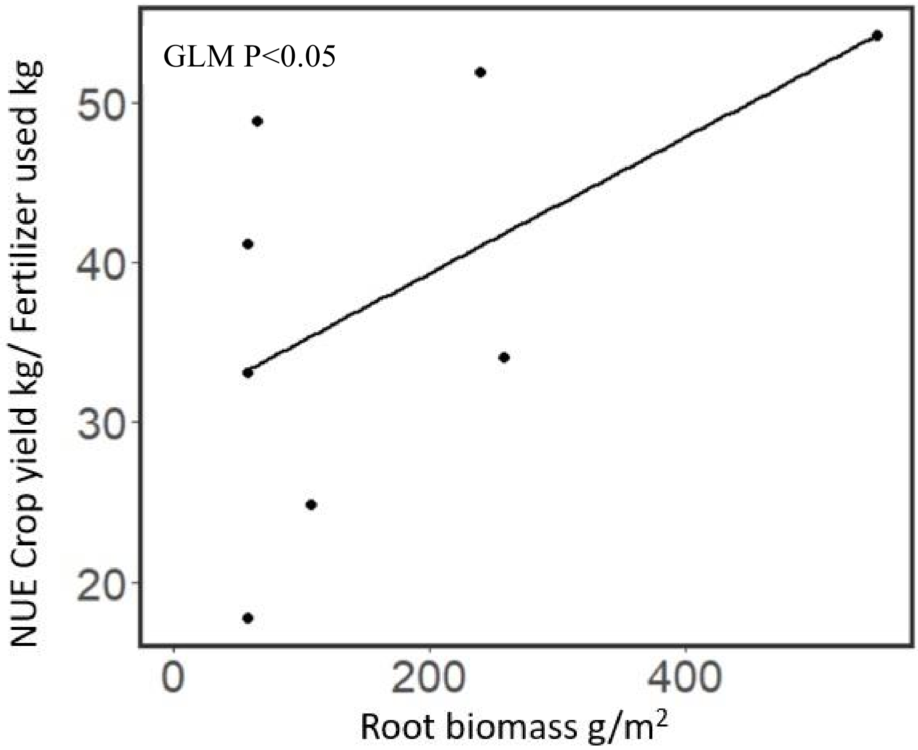

Figure 5. Correlation between Nitrogen Use Efficiency (PFP_N) and root biomass of TF and NF combined. Generalized linear model (GLM) analysis between NUE and root biomass showed significant values at $p<0.05$.

Table 1. Comparison of soil physical and chemical parameters between 4 different treatments in 3 layers.
Average values with a standard deviation of soil status sampled during the harvest of wheat (2015-06-01). Significant differences are indicated * P ≤ 0.05, ** P ≤ 0.01 and *** P ≤ 0.001 (Tukey HSD). TC=tillage and control, NC=no tillage control, TF=tillage fertilizer and NF=no-tillage fertilizer.

										Factors	
Depth	Parameters	NC		NF		TC		TF		Tillage	Fertilizer
0-5 cm	pH	6.08 (0.42)	a	5.80 (0.32)	b	6.82 (0.08)	a	6.35 (0.15)	a	***	**
	EC (mS/m)	12.5 (10.61)	a	15.38 (3.49)	a	4.37 (0.67)	b	6.42 (1.71)	b	**	
	Bulk density (g/cm^3)	0.60 (0.04)		0.54 (0.08)		0.61 (0.03)		0.53 (0.03)			
	Water content %	39.77 (4.91)		53.21(23.65)		37.14 (11.27)		49.22 (11.19)			
	C%	8.07 (1.31)	a	9.87 (1.44)	a	6.42 (0.41)	b	6.85 (0.82)	b	**	
	N%	0.60 (0.11)	a	0.78 (0.13)	a	0.46 (0.03)	b	0.50 (0.07)	b	**	
	C/N	12.59 (0.25)		12.6 (0.26)		13.98 (0.13)		13.59 (0.10)			
5-10 cm	pH	6.54 (0.37)		6.46 (0.26)		6.88 (0.09)		6.4 (0.07)			*
	EC (mS/cm)	4.1(1.54)		4.93 (1.71)		3.9 (0.49)		4.8 (1.08)			
	Bulk density (g/cm^3)	0.55 (0.13)		0.67 (0.10)		0.53(0.05)		0.55 (0.02)			
	Water content %	53.12 (9.76)		42.43 (8.23)		51(2.89)		51.31(4.63)			
	C%	5.84 (1.44)		5.4 (0.98)		6.11(0.45)		6.23 (0.98)			
	N%	0.43 (0.04)		0.37 (0.08)		0.43 (0.04)		0.44 (0.06)			
	C/N	13.96 (0.77)		14.7 (0.79)		14.09 (0.39)		13.69 (0.32)			
15-20 cm	pH	6.76 (0.34)		6.60 (0.39)		7.03 (0.21)		6.51(0.18)			
	EC (mS/cm)	3.14 (1.60)		3.1 (0.96)		3.13 (1.12)		4.74 (1.86)			
	Bulk density (g/cm^3)	0.60 (0.09)		0.64 (0.09)		0.63 (0.04)		0.62 (0.02)			
	Water content %	48.57 (5.15)		48.71(4.97)		44.08 (13.21)		54.68 (13.78)			
	C%	5.13 (0.75)		6.10 (1.71)		5.23 (0.54)		5.25 (1.88)			
	N%	0.33 (0.06)		0.39 (0.10)		0.36 (0.05)		0.35 (0.12)			
	C/N	15.52 (0.59)		15.56 (0.4)		14.84 (1.13)		14.88 (0.63)			
	N leaching (g-N/m^2)	0.06 (0.04)		0.16 (0.03)		0.15 (0.01)		0.19 (0.02)			

Table 2. Soil macrofauna biomass (g/plot) of four treatments.

Average values of macrofauna biomass on four treatments. Significant effects are represented by * P ≤ 0.05, ** P ≤ 0.01 and *** P ≤ 0.001 (Tukey HSD). TC=tillage and control, NC=no tillage control, TF=tillage fertilizer and NF=no-tillage.

									(P-value)	
Soil Fauna (Pitfall)	NC		NF		TC		TF		Tillage	Fertilizer
Araneae	0.1439		0.1523				0.0022			
Isopoda	0.0678		0.1957		0.0042		0.0506			
Ants	0.0028		0.0051		0.0167		0.0128			
Coleoptera	0.0049		0.0983		0.0077		0.0238			
Orthopterodia	0.0032		0.003		0.0042					
Opiliones	0.0025		0.0042		0.0002		0.0048			
Lepidoptera			0.0108							
Amphipoda			0.0044				0.0037			
Gastropod										
Hemiptera	0.0545				0.0002		0.0515			
Diptera					0.0004					
Diplopoda							0.0138			
Chilopoda							0.0108			
Total biomass	0.2797	a	0.474	a	0.0337	b	0.1741	b	*(0.036)	(0.137)

Table 3. Nitrogen content in wheat grain and weed (g/m^2). Average values with a standard deviation of nitrogen content in the wheat grains and weeds. Means with different letters represent a significant difference among the treatments at each sampling (Tukey HSD test, $p<0.05$). TC=tillage and control, NC=no tillage control, TF=tillage fertilizer and NF=no-tillage fertilizer.

	NC				NF				TC				TF			
	Mean		S. D.		Mean		S. D.		Mean		S. D.		Mean		S. D.	
Wheat	1.11	±	0.1	a	3.88(0.4)	±	0.4	b	1.11	±	0.1	a	2.88	±	0.5	b
Weed	5.70	±	0.6	a	3.10 (0.9)	±	0.9	a	0.64	±	0.1	b	0.66	±	0	b

Table 4. Ammonium and nitrate (g/m^2) extracted from Ion exchange resin (IER).
Means with different letters represent a significant difference among the treatments at each sampling (ANOVA, $p<0.05$).

Treatment	Ammonium	S. D.		Nitrate	S. D.		Total inorganic N	S. D.	
TC	-0.01	0.01	a	0.05	0.01	a	0.04	0.01	a
TF	0.002	0.01	a	0.13	0.05	b	0.14	0.05	b
NC	-0.02	0.01	a	0.01	0.003	a	-0.01	0.007	a
NF	-0.01	0.01	a	0.13	0.02	b	0.12	0.02	b

Chapter 5

General Discussion

This thesis demonstrated the potential of no-tillage with weed (NTW) practice as sustainable resource management in the agriculture system. Living weeds are the source of N in the agriculture field that has the potential to release N to the soil and improve the nitrogen use efficiency in crops. This study demonstrates the importance of the NTW system for the conservation of soil organic matter and soil animals and shows how small-scale tillage within one year can immediately degrade the soil ecosystem. Furthermore, the results showed that the fertilizer is efficiently used in the NTW system compared to the tillage system which was estimated by calculating the nitrogen use efficiency (NUE). In this chapter, I discuss the impact of NTW practice on conservation of soil organic matter, soil animals and better nutrient recycling by using weeds, without using external resources.

Soil pH, EC and bulk density changes in NTW

Soil physical and chemical properties govern various soil processes like organic matter decomposition, soil animal activity, and nutrient cycling in the agriculture system. The implementation of NTW did not change the soil pH, EC, and bulk density drastically in this study. The significant decrease in pH was observed only when fertilizer was used (Chapter 4, Table 1). Fertilization may decrease the pH due to the potential nitrification of added fertilizer and increasing the acidity of the soil (Pascual et al., 2007; Savci, 2012). NTW promoted the root growth in this study and higher root biomass have shown to increase in soil organic material, which causes an increase in soil humidity and a decrease in soil pH (Arvas et al., 2011; Citak and Sonmez, 2011).

Long-term studies that were conducted for 10 years have found that no-tillage reduces the bulk density and improves soil porosity, moisture, organic matter accumulation, and better plant root growth (Bai et al., 2009; He et al., 2011). A 3 years study conducted by Logsdon and

Cambardella 2000 found no significant variation in soil bulk density between the tillage and no-tillage treatments, which may be due to the short-term duration of the experiments. Cover crop and mulching has shown to decrease soil compaction by increasing the soil moisture, modifying the temperature and increasing the soil organic matter in long-term experiments (Blanco-canqui and Claassen, 2011; Stirzaker and White, 1995). NTW is independent of heavy soil disturbance and uses organic mulch to conserve the soil. Hence, long-term adoption of NTW might improve soil physical properties by decreasing the soil bulk density due to presence of weed mulch.

Soil carbon and nitrogen changes

Conservation agriculture (CA) system strives to conserve the soil organic matter by application of its three core principles (Hobbs, 2007; Mloza-Banda et al., 2016; Palm et al., 2014; Puget and Lal, 2005). While most CA practice uses external source for mulch such as straw mulch and cover crops to conserve the soil (Ciaccia et al., 2015; Radicetti et al., 2016; Vance et al., 2014), NTW uses weeds that are available within the field (Kaneko, 2014). NTW showed the potential to increase the soil organic matter by increasing the root biomass and weed mulch. The feasibility of weed as N source for soil was validated in this study, when the carbon and nitrogen concentration increased as the amount of weed mulch was increased. This thesis also demonstrated the impact of tillage on the soil organic matter. Generally, accumulation and increasing the soil organic matter can take 5-10 years after the introduction of no-tillage (He et al., 2011a; Rusinamhodzi et al., 2011). But performing tillage on a long-term NTW farm immediately degraded and reduced the soil organic matter within one year. N leaching was similar in tillage and no-tillage treatment. But the total soil carbon and nitrogen were significantly lower in tillage treatment, which means that N loss may have occurred through different paths such as runoff, volatilization, or erosion. Hence, NTW can be considered as a sustainable soil conservation practice, which can conserve and increase the soil organic matter without using external resources.

Soil fauna

Soil fauna plays an essential role in maintaining the soil ecosystem. The tunneling and burrow activity of soil fauna increases the porosity of the soil, increases water infiltration, and reduces runoff. They also mix the litter and decompose the complex organic matter, influencing the nutrient cycling in the soil ecosystem. Researches have shown that plant diversity in the grassland ecosystem impacts the microbial biomass due to increases in plant production and species richness, which results in complex food web structure (Lange et al., 2015b; Loranger-merciris et al., 2006; Zak et al., 2014). Other studies argue that rather than plant productivity, the amount and quality of resource entering the decomposer system affects the soil microbial community as the decomposer system provides essential ecosystem services such as litter decomposition and nutrient mineralization (Eisenhauer et al., 2010). The presence of weeds in the NTW system increases the above-ground plant diversity and slashing and mulching increase the available resources for the soil organisms. NTW represents a combination of grassland and agriculture system and its effect on microbial activity is demonstrated. NTW practice can increase the soil microbial activity due to conservation of root and surface organic matter. Roots can act as nutrient resource and also release roots exudates that can increase the soil microbial activity (Lange et al., 2015a). This study also showed the lower microorganism activity when the soil was tilled. The introduction of tillage has been documented to reduce soil microbial biomass due to physical disturbance to the soil and loss of nutrients (González-Chávez et al., 2010; Mathew et al., 2012). Here, NTW showed the potential to conserve the soil microbial biomass while reduction in tillage practice was observed. The highest microbial biomass was observed in chapter 2 (Table 1), which suggests that long-term adoption of NTW may increase soil microbial biomass. NTW might be able to bridge grassland and agriculture systems, where soil biota in the agriculture ecosystem can be conserved using principles of grassland ecosystem (Klopf et al., 2017).

Macrofauna, including earthworms, functions as keystone organisms for the decomposition process and has been found to contribute to increases in primary productivity with increasing plant diversity due to heterogeneous mixture of litter (Milcu et al., 2008). Total macrofauna biomass was 17% higher in NTW treatment in chapter 2 compared to tillage treatment. The addition of fertilizer in chapter 3 showed 36% higher macrofauna biomass in NTW than tillage treatment. The higher biomass in chapter 3 might be due to an increase in nutrient availability after fertilization. NTW also showed significantly higher root biomass, which can increase macrofauna biomass due to root-derived resources. However, studies have shown that an increase in macrofauna, including earthworm, might increase due to the quality of rhizodeposits rather than quantity (Milcu et al., 2006). In our study, roots were not separated according to the plant species. But NTW did show different weed species composition compared to tillage treatment, which might have influenced the macrofauna biomass. In chapter 2, tillage reduced the soil surface litter amount and root biomass, which may have reduced earthworm biomass. Therefore, NTW can increase the soil macrofauna activity because mulching weeds on soil surfaces creates a favorable environment by increasing the soil organic matter.

Weed nitrogen release in NTW

Generally, leguminous plants are used as green manure or cover crop to release N to the soil. Hairy vetch, crimson clover, and ryegrass have shown to contribute 70-95, 37-60, and 20-24 kg N /ha to the soil, respectively (Parr et al., 2011; Ranells and Wagger, 1996; Sainju et al., 2005). The C: N ratio of leguminous plants are lower and are preferred as cover crops because they can quickly release high N to the soil. An eight weeks decomposition study showed that 3-4 t/ha hairy vetch released 95 kg N /ha and 3 t/ha crimson clover released 60 kg N /ha while 3 t/ha rye released least amount, i.e., 24 kg N /ha back to the soil (Ranells and Wagger, 1996; Wagger, 1989). Other researches with 2.7 t/ha common bean and 4 t/ha faba bean showed 32 kg N /ha and 50 kg N /ha release (Dabin et al., 2016; Etemadi et al., 2018). In these experiments, inoculants, external fertilizer and cover crops were used to increase the N fixation, external N fertilizer to increase the efficiently of cover crops. The N release depended on the fertilizer application and other factors like soil type, moisture, temperature, and type of cover crops, which might have resulted in different N release pattern in these studies.

This study demonstrated that non-leguminous weeds present within the agriculture land can contribute/release N to the soil without any external input (Table 2). Although the N release from the weeds was lower in comparison to regular leguminous cover crops, weeds contributed some N to the soil. In 5 months, N release estimated by using *I. cylindrica* in NTW was 1.7 kg/ha and 2 kg/ha in chapter 2 and chapter 3, respectively. The difference in N release might be due to the difference in the season of the experiments. N release in chapter 2 was measured in the winter season from September-February, while chapter 3 was measured in summer from June-October. After 5 months in the field, the remaining dry weight of *I. cylindrica* in the litterbag was 4.8 g and 3.8 g in chapter 2 and chapter 3, respectively, which suggest that decomposition was slower in chapter 2 study. The chopped mixed weed used in chapter 2 was quick to release 20 kg/ha of N at the same time. From the observed results, 52 ton/ha and 110 ton/ha of *I. cylindrica* mulch is required to release 100 kg/ha of N in summer and winter, respectively (Table 2). The amount of weed needed is too large and might be impractical. However, chopped mix weed might be more feasible because 9 ton/ha of weed is required to release 100 kg/ha of N in the winter season. The weed amount required may be much lower in the summer season. The amount of weeds that was available in chapter 2 site was about 2 ton/ha, which is still not enough to release 100 kg/ha of N to the soil. A large amount of weed mulch resources is required to challenge the general fertilizer usage or leguminous cover crops. But weed mulch practice demonstrated in this thesis is independent of external resources and reduces labor for N management practice compared to the conventional system. However, alternative methods such as combination of weed mulch and reduced fertilizer or compost can reduce fertilizer consumption in the agriculture lands. Through this estimation, the use of chopped mixed litter is recommended as a nitrogen input method in the NTW system to reduce external input.

Nitrogen use efficiency (NUE) in NTW

NUE is an important index in agriculture land as they show the efficiency of fertilizer applied and shows the loss of nutrient and yield gap. Many technologies have been developed to increase NUE, such as using high yielding cultivars, enhanced fertilizers, cover crops, and inoculants to increase N uptake. However, these require investment in external resources and are only applicable in certain areas that have access to these technologies, which might explain the increase of only 2% world's NUE in the past two decades (Omara et al., 2019). This study demonstrates an alternative method to increase NUE in agriculture farms without the use of external resources using NTW practice.

In chapter 4, the NTW system was more efficient in nitrogen utilization than the tillage treatment (Chapter 4, Fig. 2). The agronomy efficiency (AE_N) (NTW = 32.17, tillage = 22.62 kg/kg) and crop recovery efficiency (RE_N) (NTW = 0.55, tillage = 0.35 kg/kg) for NTW was 40 % and 50% higher than tillage treatments respectively. The average world AEN and REN for cereal crops was estimated at 30 kg/kg and 0.35 kg/kg (Cassman et al., 2002; Dobermann, 2005). The results showed higher NUE in NTW compared to tillage, which means that crops in NTW utilized more N and minimized the loss to the environment. The NUE difference in NTW and tillage is large and considering the world average, the tillage practice might have reduced the NUE due to loss of nitrogen from the soil.

In last 20 years, the USA increased their overall NUE by 10% and has the highest average cereal NUE (41%) in the world, evaluated in 2015. On the other hand, India has the lowest NUE at 21% (Omara et al., 2019). The gap of NUE between the two countries shows that the USA had better technologies such as improved cultivars and precision crop management to improve the NUE resulting in reduced fertilizer usage, but India was not able to utilize or access these technologies and relied mostly on heavy fertilizer use, which decreased the NUE (Omara et al., 2019). NTW practice demonstrated in this thesis is independent of external input and utilizes locally available resources and is applicable in every part of the world. NTW treatment showed potential to increase NUE by promoting the root growth and may be appropriate nitrogen management alternative specially in resource-poor areas. However, challenges like weed control, yield lost during early adoption, technical aspect of management practice, and resource material for mulch needs to be considered while adopting the NTW practice. A prior study of the farm on soil condition, weed community structure, and resource availability can assist in the effective adoption of the NTW system. This study shows the potential of NTW to maintain the crop production similar to conventional tillage treatments due to efficient NUE in the presence of weeds.

Weed management in NTW

The dual properties of weeds in the NTW management system are demonstrated in this study. First, which is more of a passive stage, where the weeds are grown in the agriculture field that does not require any management, however, some exceptions, such as fast-growing and invasive weed species, should be identified and removed from the farm. During the passive stage, the weeds increase the plant diversity and simultaneously act an N sink. Second, the weeds are slashed and used as a mulching resource for the soil. The weeds are slashed without picking the roots, which can either uptake the N from the soil for growth or act as an organic matter for the soil. The weed slashing was done manually by using sickle in chapters 3 and 4, while in chapter 2, a hammer mower was used. Initially, the weed is slashed before the seeding. At the later stage, the weeds are slashed depending on the growth of weeds, which depends on various factors such as the height of the weeds and the seasons. The experiment in chapter 4 was carried out on the winter season and slashing the weeds one time before seeding was enough to control the growth of the weeds. In chapter 3, the slashing frequency of weeds was evaluated in the summer season. After the initial slash before seeding, slashing weeds once was enough to gain the highest crop yield. The slashing of weeds was done one month after the seeding when the weeds and crop height was similar. Generally, plants at higher height can compete with plants with lower height as they have an advantage over capturing sunlight, nutrients, and other resources. Hence, slashing is recommended before the weeds grow higher than the crops.

Concluding remarks

Tillage practice degraded the soil ecosystem and may not be feasible for efficient nitrogen management. Conversely, this thesis showed the potential of NTW practice towards the development of a nitrogen efficient agriculture practice by using the weeds. Indeed, weeds in agriculture land need to be controlled, but the studies demonstrated in this thesis are a small step towards developing a sustainable weed management without using external resources. Weeds act as a source of nitrogen that can reduce the dependency of external N based input in the agricultural system. Weed slashing and mulching contributed N to the soil and reduced the competition between the crops and weeds. Even with the presence of weeds, the crop in NTW practice was able to efficiently utilize the added fertilizer with similar yield as tillage practice due to higher soil N pool. This shows that NTW is one of the ways to improve nitrogen use efficiency in conservation agriculture practice.

NTW practice is independent of heavy machinery, external input, and requires less labor and can potentially be useful for small-scale farmers in marginal environments. Less dependency on external inputs and machinery, such as chemical fertilizers and herbicides, can be economically beneficial for farmers and also conserve the environment by reducing nutrient loss. Furthermore, adoption and further research on NTW practice first on a small scale can assist in expanding NTW to large scale agriculture.

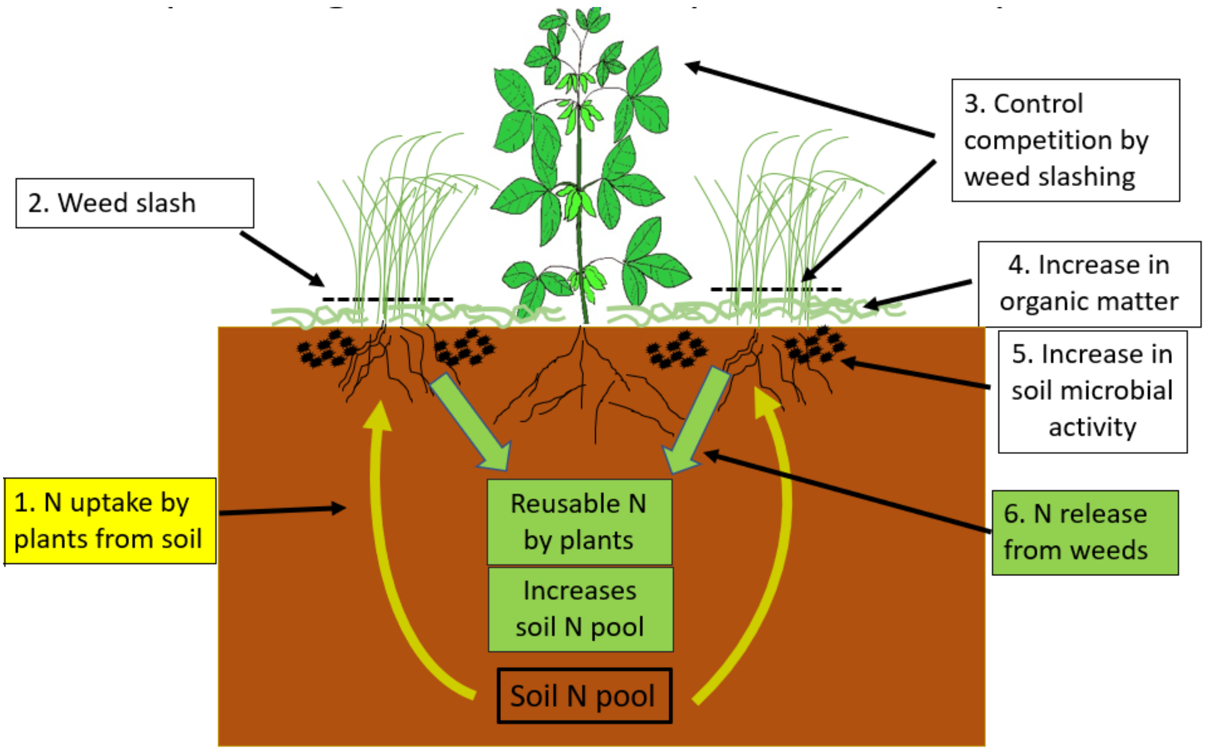

Figure 1. Conceptual diagram illustrating the N release by weeds in the NTW system.
The weeds growing beside the crops are slashed and mulched on the soil surface. The weed slash and mulch practice reduced the competition between the weeds and crops and also contributed towards the soil organic matter. The addition of weed mulch on soil increased the soil organism's activity that decomposes and then releases N from the organic matter to the soil. The released N is again used by weeds and hence creates an N cycle in NTW.

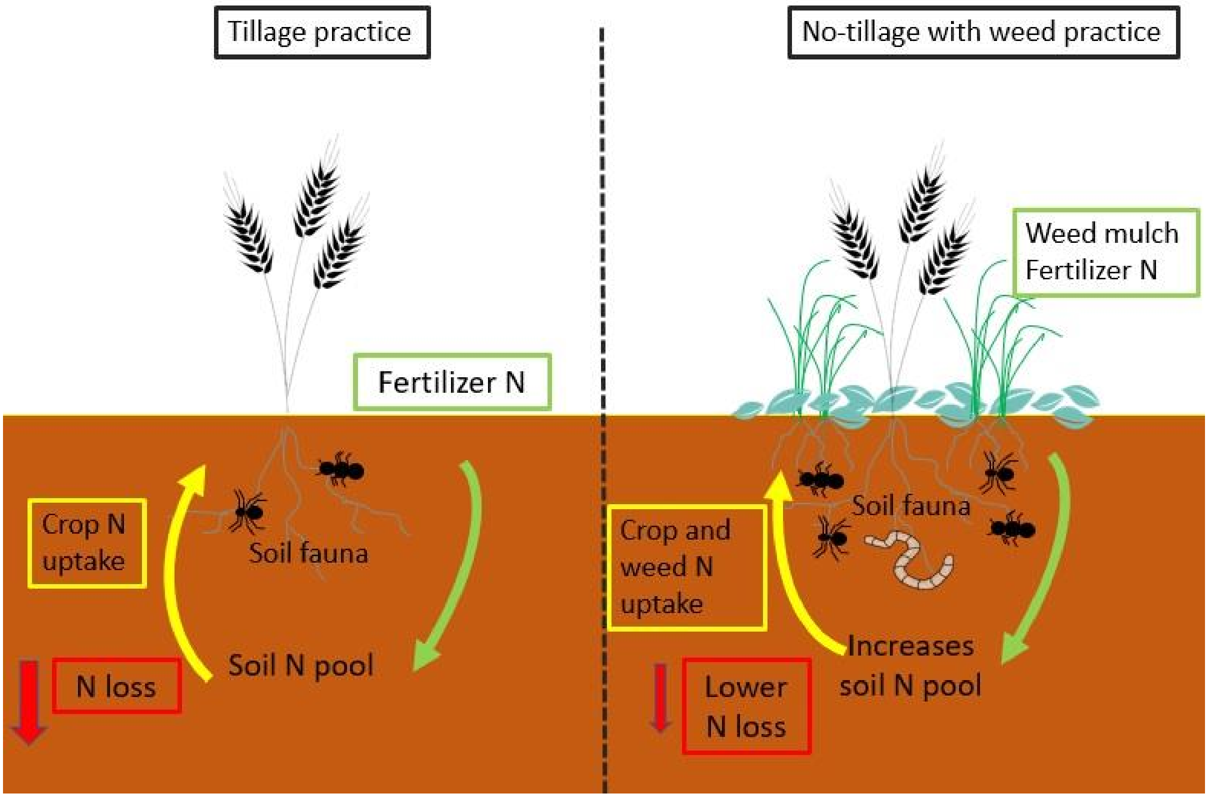

Figure 2. Conceptual diagram illustrating nitrogen use efficiency (NUE) between tillage and no-tillage with weed (NTW) practice. NTW system utilizes weeds in the agriculture field. Weeds increase plant diversity and can uptake excess nitrogen, which may not be accessible to crops. So, weeds have the potential to remove nutrients from the soil, reducing the loss, but they still compete with crops for the resources. In the NTW system, these weeds are slashed and mulched, which hosts soil animals and returns the nutrient to the soil pool that can again be used by the crops or weeds that creates a closed nutrient system that conserves the nutrient within the system reducing the loss.

Table 1. Total soil microbial biomass (nmol/g) in all three studies presented on this thesis.

Chapter	Treatment	Total microbial biomass		
		Mean		S. D
Chapter 2	NT	128.9	±	16.1
Chapter 3	S0	38.5	±	3.9
	S1	71.6	±	5.1
	S2	68.7	±	6.5
Chapter 4	NC	74.3	±	9.9
	NF	54.2	±	10.5
	TC	41.7	±	4.0
	TF	43.5	±	4.9

Table 2. Estimation of weed required for mulching purposes

Chapter	Treatment and material	Amount of weed used in this study	N released in this study (Duration 5 months)	Amount of weed required to release 100 kg/N (Duration 5 months)
2	No-tillage chopped mixed mulch	1.9 ton/ha	20 kg/ha	9 ton/ha
2	NTW with *Imperata cylindrica*	1.9 ton/ha	1.7 kg/ha	110 ton/ha
2	Tillage with *Imperata cylindrica*	1.9 ton/ha	0.5 kg/ha	325 ton/ha
3	NTW with *Imperata cylindrica*	0.9 ton/ha	2 kg/ha	52 ton/ha

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