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4 1 Optimizing the harvesting stage of rye as a green manure to maximize nutrient production and to
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6 2 minimize methane production in mono-rice paddies
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Abstract

Rye (*Secale cerealis*) has been widely cultivated to improve soil quality in temperate paddies. However, its biomass incorporation can significantly increase greenhouse gas emissions, particularly the emission of methane (CH₄), during rice cultivation. The chemical composition and productivity of cover crop biomass may vary at different growing stages. Therefore, nutrient productivity and CH₄ production potential might be controlled by selecting the optimum harvesting stage. To investigate the effect of rye harvesting stage on nutrient productivity and CH₄ production potential, rye was harvested at different growing stages, from the flowering stage to the maturing stage, for seven weeks. The chemical composition and biomass productivity of rye were investigated. CH₄ production was measured by laboratory incubation, and CH₄ production potential was estimated to determine the real impact on CH₄ dynamics in rice soils. Rye biomass increased with plant maturation, but nutrient productivities such as N (nitrogen), P₂O₅, and K₂O were maximized at the flowering stage. The contents of cellulose and lignin increased significantly as plants matured, but the total N, labile organic carbon (C), and hot and cold water-extractable organic C clearly decreased. Soils were mixed with 0.3% (wt wt⁻¹ on dry weight) air-dried biomass and incubated to measure the maximum CH₄ productivity at 30 °C under flooded conditions. Maximum CH₄ productivity was significantly correlated with increasing labile organic C and protein content, but it was negatively correlated with total organic C, cellulose, and lignin content. CH₄ production potentials were significantly increased up to the pre-maturing stage (220 DAS) and remained unchanged thereafter. As a result, CH₄ production potential per N productivity was the lowest at the late flowering stage (198 - 205 DAS), which could be the best harvesting stage as well as the most promising stage for increasing nutrient production and decreasing GHG emissions in temperate mono-rice paddy soils.

Keywords: cover crop, greenhouse gas, labile organic carbon, nutrient productivity, *Secale cerealis*

1. Introduction

Cover crops are usually grown to cover the ground and protect the soil from erosion and loss of plant nutrients through leaching and runoff (Reeves, 1994). Because crop residues are mostly removed for livestock feeding in many Asian regions, cover crop cultivation during the fallow season is strongly recommended to improve soil quality. In temperate countries such as Korea, the winters are characterized by long, dry and cold weather. Winter cover crops that can tolerate such harsh weather conditions are critically important (Haque et al., 2013). The main cover crops include barley, hairy vetch and rye. Winter rye is considered the most viable in this region, due to its high tolerance against drought and extreme weather conditions (Kim et al., 2013). Another advantage of rye is its high biomass yield compared to other cover crops. It grows rapidly and is adapted to low temperatures, so it provides a higher input of organic matter into the soil (Kim et al., 2013) and can be an excellent source of animal feed when other green forages are not available (Oelke et al., 1990). In addition, rye is known to have a very strong and long root system that can hold the soil in place, thus preventing soil erosion and loss of nutrients (Smith, 2011).

The mineralization of organic substrates controls the availability of nutrients to the field crops, and soil microorganisms play an important role in determining the mineralization rate of organic matter. The kinetics of decomposition of organic substrates in soil and their C and N mineralization could be strongly influenced by the quality of the materials (Heal et al., 1997). It is quite clear from the literature that N content or the C/N ratio of green manures is the principal determinant of biomass application effects on soil N availability after incorporation into soil (Hargrove, 1986; Smith et al., 1987; Ranells and Waggoner, 1996). Therefore, the C/N ratio of cover crop biomass could be a crucial factor to consider in determining the value of green manure because it affects the nutrient content of the soil and may starve a crop of N if the incorrect plants were used as a green manure.

Compared to leguminous plants such as hairy vetch, rye biomass has a low N content and a high C/N ratio. Thus, its residue as a green manure could have some undesirable characteristics that may interfere with planting and growth of the succeeding crop in the short term. The C/N ratio of plant biomass

will differ from species to species, depending on age. As plant tissue N increases or tissue C/N ratio decreases, the initial N mineralization potential and N mineralization rate increase (Frankenberger and Abdelmagid, 1985; Kuo and Sainju, 1998), and the crossover time for net N mineralization decreases (Kuo and Sainju, 1998). The tissue C/N ratio of cover crops generally decreases in the vegetative growth stage, is minimized at the flowering stage, and thereafter significantly increases with maturity.

Similar results were obtained by Dalal et al. (2008), who found that methane (CH₄) production increased because of the high C/N ratio of rye cover crop application. Because the productivity and the tissue C/N ratio of cover crop biomass can be affected by the growing stage, control of the harvesting time might be an important management technique for cover crops to increase nutrient productivity and availability.

However, using biomass as a green manure could greatly increase the emissions of greenhouse gases (GHGs) such as CH₄ and CO₂ in arable soils. CH₄ is mainly produced from the decomposition of organic matter by anaerobic archaeal methanogens under extremely reduced conditions (Garcia et al., 2000). Cover crop biomass greatly stimulates CH₄ emissions in flooded paddy soils (Kim et al., 2012; Kim et al., 2013; Haque et al., 2013). CH₄ production from organic substrates mainly depends on substrate availability. The composition and biodegradability play a crucial role in CH₄ production from energy crops.

For example, lignocellulose in plant biomass consists of three biopolymers, cellulose, hemicellulose and lignin, which have a remarkable influence on CH₄ production (Vindis et al., 2010). CH₄ productivity shows a strong positive correlation with labile Cs such as water extractable carbon (C) and hot water extractable C, as well as total N, because rye cover crop application has the highest potential of soil organic C accumulation, which alternatively increases CH₄ emission (Denef et al., 2009). In contrast, CH₄ productivity shows a highly negative correlation with cellulose, hemicellulose, lignin and polyphenol content. These results are similar to those of Kazakou et al. (2009), who stated that decomposition of organic constituents increases with increasing concentrations of cellulose, hemicellulose and lignin, which alternatively will reduce CH₄ productivity. Because the composition of lignocellulose and other organic

chemicals in biomass could be markedly different during different cover crop growing periods, the choice of harvest time could be important for reducing CH₄ emissions in rice paddy soil.

We hypothesized that the organic components of the rye cover crop change greatly across growth stages in a way that can influence nutrient availability in the soil as well as CH₄ production in the flooded paddy soil. The aim of this study was mainly to determine whether rye biomass could be harvested at particular stages to mitigate CH₄ production and to enhance nutrient production in paddy soil. In this study, the effects of harvesting time and incorporation of rye cover crop biomass on CH₄ production and nutrient production in rice paddy soil were evaluated to determine the optimum season for rye harvesting in both field and laboratory-scale experiments. For this purpose, winter rye was harvested at different growing stages, and CH₄ production potentials and nutrient input of the biomass were estimated.

2. Materials and methods

2.1. Rye cultivation and harvesting

Winter cover crops were cultivated normally in the late autumn and harvested in the following spring. The cover crop was then incorporated as a green manure to improve the soil quality before rice cultivation in summer. In this study, rye (*Secale cerealis*) seeds were sown at the recommended seed rate (120 kg ha⁻¹) (Kim et al., 2013) after rice harvest in mid-October 2010 in a rice field (Naeback ri, Sudong myeon, Hamyang; South Korea; coordinates 35° 36' 03.56" N, 127° 48' 07.50" E). The soil in this experimental site belongs to the Pyeongtaeg series (fine-silty, mixed, nonacid, mesic Typic Endoaquept) (USDA, 2010). The soil organic C content was 9.0 ± 0.9 g kg⁻¹, and the soil pH was 5.9 ± 0.3 (1:5 with H₂O) before the cover crop incorporation. The field (40 m x 25 m, 1000 m²) has been managed under the same farming practices for a rye cropping system over the past several decades. Rye was cultivated without adding any chemical fertilizer and maintained under upland conditions during cultivation. Based on maturation time, the above-ground biomass from three 1-m² areas within the experimental field was harvested every week

from 191 DAS (days after rye seeding) to 233 DAS, which has typically been recommended for harvesting and incorporating green manure in rice fields (Kim et al., 2012; Kim et al., 2013). The whole above-ground biomass was air-dried, the dry weight was recorded.

2.2. Measurement and estimation of CH₄ production

Soil samples were collected from the plough layer (0 - 15 cm) before rye seeding in the same field, and the samples were air-dried and sieved (< 2 mm). To evaluate the feasibility of using rye harvested at the different maturity stages, five grams (5 g) of dry soil were thoroughly mixed with 0.14 mg (0.3% *wt wt*⁻¹, roughly corresponding to 5 Mg ha⁻¹ dry weight under field conditions) ground rye powder (40 mesh), and then transferred to a 125 ml serum bottle. The same amount of rye was incorporated during the laboratory-scale incubation, which made it possible to standardize conditions and consider only the changes in chemical composition of rye biomass, avoiding confounding with other environmental variables such as temperature and water availability. Deionized water (20 ml) was added to the serum bottle, which was sealed with a butyl rubber stopper and flushed with oxygen-free N₂ (nitrogen) gas for 30 min. The bottles were incubated at 30 °C in the dark for 45 days. Gas sampling was carried out every 3-5 days (12 times) from the same bottles, and changes in CH₄ concentrations in the headspace were monitored by a gas chromatograph (GC-2010, Shimadzu, Kyoto) equipped with a packed Porapak Q column (80-100 mesh) and a flame ionization detector (FID). The temperatures of the column, injector and detector were adjusted to 100 °C, 200 °C and 200 °C, respectively. Helium (He) and hydrogen (H₂) were used as carrier and burning gases, respectively.

The CH₄ production potential was estimated by using the following equation. Rye biomass and CH₄ production were simultaneously considered to estimate CH₄ production potential in realistic field conditions because rye productivity could vary significantly depending on harvesting season. The estimated CH₄ production was expressed as g CH₄ produced ha⁻¹ as follows:

Estimated CH₄ production (g CH₄ ha⁻¹) = [CH₄ production potential (g CH₄ kg⁻¹ dry soil) / rye used during incubation (g of dry rye kg⁻¹ dry soil)] x rye productivity (g of dried rye biomass ha⁻¹).

2.3. Chemical composition of rye

The air-dried rye samples were ground and homogenized to pass through a 0.1 mm sieve for chemical analysis. Total C and nitrogen (N) contents were determined with a CNS elemental analyser (CHNS-932 Analyzer, Leco, USA). Labile C and N, such as cold water extractable C and N (CWE C and CWEN) and hot water extractable C (HWE C and HWEN) (Ghani et al., 2003) were evaluated by using a total organic CN analyser (TOC-VCPN, Shimadzu, Kyoto). Hemicellulose, cellulose and lignin were measured with a slightly modified method described by Sjöberg et al. (2004). Using acid hydrolysis with 72% H₂SO₄, dilution and refluxing with dilute acid (Berg et al., 1982; Bethge et al., 1971), an insoluble organic residue (Klason lignin) was obtained. Hemicellulose was determined as the sum of arabinose, xylose, mannose and galactose, whereas cellulose was calculated as the total amount of glucose. The HPLC analysis was previously described in detail by Sjöberg et al. (2004).

2.4. Statistical analysis

All data were analysed using one-way analysis of variance (ANOVA) with SAS 9.1 (SAS Institute Inc., Cary, NC). Tukey's honest significant difference (HSD) test was used to test for differences when the F-test was significant at the $P < 0.05$ probability level. Linear regression and correlation tests were performed to evaluate the relationships between response variables.

3. Results

3.1. Changes in rye chemical composition and biomass with growth

The chemical composition and biomass of rye changed significantly during the maturation season. Total organic C (TOC) decreased slightly but continuously with plant growth, from 411 g kg⁻¹ on the 191st day to 402 g kg⁻¹ at the last sampling on the 233 DAS, but there was no major difference. In comparison, total N content was very high during the mostly vegetative stage, 35.1 g kg⁻¹ at 191 DAS and 34.7 g kg⁻¹ at 198 DAS, but it decreased significantly with plant maturity, reaching 0.41 g kg⁻¹ at the last sampling stage (233 DAS). As a result, the C/N ratio significantly increased with rye maturity. The C/N ratio of the biomass was very low, ranging from 12 to 18 over the course of 191 to 205 DAS. Thereafter, it significantly increased with plant maturation and reached a maximum of 101 at the last stage (233 DAS). As with total N content, nutrient contents such as P₂O₅ and K₂O showed the highest levels at the early growing stage (191 - 205 DAS) and continually decreased with plant growth from 21, 4.9 g P₂O₅ kg⁻¹ on the 191st day to 8, 2.1 g K₂O kg⁻¹ at the last sampling (233 DAS), respectively.

Unlike the changes in TOC content, the labile organic C contents such as C_{WEC} and H_{WEC} decreased significantly with rye maturation (Table 1). The proportions of C_{WEC} and H_{WEC} to TOC were 5.2% and 6.5% at 191 DAS, respectively, but decreased to 1.6% and 1.9% at 233 DAS. As with total N content in rye biomass, labile N contents such as C_{WEN} and H_{WEN} significantly decreased as plants aged (Table 1). The proportions of C_{WEN} and H_{WEN} to total N were 3.0% and 3.9% at 191 DAS, respectively, but they did not significantly change by 212 DAS. Interestingly, these proportions were increased to 14% and 21% at 219 DAS, respectively, and they slightly decreased with plant age to 9.8% and 14.6% at 233 DAS.

The cellulose and lignin contents of rye biomass significantly increased with rye growth (Table 1). The cellulose content was 20% at 191 DAS, and it increased to 28% at 233 DAS. Lignin content was 16-17% at the initial stage of growth (191 - 198 DAS), and it significantly increased to 28-29% at the maturing stage (226 - 233 DAS). Hemicellulose content fluctuated in the range of 14 to 17% but did not change significantly during the growing period.

Rye biomass productivity significantly increased with plant growth compared to the initial harvesting stage (191 DAS). It reached a maximum of 7.2 Mg ha⁻¹(dry weight base) at 226 DAS, and thereafter slightly decreased to 7.0 Mg ha⁻¹(Table 1).

3.2. Changes in CH₄ production potentials

Controlling the harvesting season of rye significantly affected CH₄ production and its lag phase during the incubation (Fig. 1). Interestingly, a linear increase in CH₄ production was observed until approximately 30 days of incubation in soils treated with biomass obtained at 191 DAS, after the lag phase. However, CH₄ production usually ended within 15 days of incubation in soils treated with older rye biomass (198 - 233 DAS).

Total CH₄ production significantly decreased with rye maturity (Fig. 1 and Table 1). For example, CH₄ production was highest in soils treated with the youngest biomass (191 DAS, mean 5.4 g kg⁻¹ dry soil), and it was lowest in the oldest biomass (233 DAS, mean 1.1 g kg⁻¹ dry soil). Total CH₄ production showed significant differences between the periods of 191 - 198 DAS and 212 - 219 DAS.

3.3. Estimated CH₄ production potentials and possible nutrient productivity

CH₄ production potential was estimated by multiplying rye biomass productivity and total CH₄ production of the biomass-treated soil to extrapolate the potential CH₄ production in realistic rice paddy conditions (Fig. 2). We confirmed that rye harvesting season significantly affected CH₄ production potential in rye-treated rice soils. CH₄ production potential was estimated to be 743 kg CH₄ ha⁻¹ (191 DAS) in rye-treated soil, which showed the lowest value among all treatments. In contrast, CH₄ production potential increased significantly to 1334 - 3348 kg CH₄ ha⁻¹ in soils treated with rye harvested at 198 to 233 DAS compared to 191 DAS due to increasing rye biomass and decreasing total CH₄ production.

The nutrient productivity of rye biomass as a green manure was evaluated in terms of biomass N productivity (kg N ha^{-1}). Rye biomass production was multiplied by the total N content of the biomass to estimate the potential N input into rice soils. Biomass N production increased with plant age, reaching its maximum from 198 to 205 DAS (106 kg N ha^{-1}), and thereafter it drastically decreased with rye maturity to $28.9 \text{ kg N ha}^{-1}$ at 233 DAS (Fig. 2). Other nutrients, such as P_2O_5 and K_2O , showed similar patterns of change.

To select the best harvesting stage for maximizing nutrient production and minimizing CH_4 impact, CH_4 production potential per unit N input from biomass was considered (Fig. 2). The value of CH_4 per N production gradually increased with rye maturity, reaching its lowest value ($13 \text{ kg CH}_4 \text{ kg}^{-1} \text{ N}$) at 191 DAS.

4. Discussion

4.1. Minimizing CH_4 production by controlling the rye harvest season

Cover crop management has been strongly recommended in mono-rice cultivation systems to improve soil quality. Application of green manure is a useful soil management practice because it can maintain soil organic matter and reduce dependence on mineral fertilizers (Elfstrand et al., 2007). However, cover crop incorporation significantly increases CH_4 emissions in rice paddies during cultivation (Haque et al., 2013; Kim et al., 2012; Kim et al., 2013). Therefore, it is desirable to find the ways to minimize CH_4 emissions from rice paddy soils treated with green manure such as rye.

Our previous field studies showed that it is possible to mitigate CH_4 emissions from rice paddies by selecting particular cover crops or adjusting the application level (Kim et al., 2012; Kim et al., 2013; Haque et al., 2013). However, in these studies, the cover crops were harvested only a week before rice seedling transplantation and then applied to rice fields. The chemical properties of plant biomass, including cellulose and lignin content, can change as the plant matures (Carpita, 1996; Nizami et al., 2009), so the rye harvest season may influence the CH_4 dynamics in rice soils treated with rye biomass. This is due to changes in the C and N composition in the rye biomass. In this study, we clearly showed

the feasibility of regulating CH₄ production by controlling the harvest season of the cover crop (Fig. 2 and Table 1).

Interestingly, we found that chemical concentrations in the cover crop biomass varied greatly with growth stage (Table 1). In particular, total organic C, C_{WEC}, and H_{WEC} in the cover crop biomass changed significantly depending on the harvesting period. These carbon sources could be important substrates for CH₄ production in the soil (Lu et al., 2000; Le Mer and Roger, 2001; Ghani et al., 2003), and they show positive correlations with total CH₄ production (Table 1). In this study, we observed that CH₄ production is typically high in rice soils treated with rye harvested at an early stage of growth. This indicates that early incorporation of rye biomass as a green manure can stimulate greater CH₄ production, due to high C availability. Some of the relevant variables have significant negative correlations with total CH₄ production, including the C/N ratio and levels of cellulose and lignin. In particular, lignin is the most recalcitrant part of the structural carbohydrates in plant biomass because it is insoluble in water and resistant to microbial action, and oxidative forces (Lewis and Davin, 1998; Hendriks and Zeeman, 2009). Therefore, inefficient decomposition of rye biomass results in reduced CH₄ production at the late stage of the rye harvesting season, which might limit the CH₄ production rate in rice paddies treated with cover crop biomass. Therefore, controlling the harvest season can influence C availability, which mainly affects CH₄ production in rice soils treated with rye biomass during rice cultivation.

In addition, the concentrations of N, P₂O₅ and K₂O in rye biomass also decreased significantly until the late harvesting stage, which might be associated with dilution as plant biomass increases. However, the decrease in total N concentration was much greater than that of other nutrients because a considerable proportion of N in young rye biomass can be transferred to the root (Cooper and Clarkson, 1989). In this study, nutrients showed significant positive relationships with total CH₄ production, indicating that CH₄ production can be strongly driven by nutrient availability. Among nutrients, N contents, including total N, WECN, and HWEN, were found to be very important parameters that influence CH₄ production, which has also been found in other wetland ecosystems (Bodelier, 2011; Kim et al., 2015). Therefore, controlling harvest season can also affect nutrient availability, which may regulate CH₄ dynamics in wetland rice ecosystems.

4.2. Optimum harvest season to minimize CH₄ and enhance nutrient productivity

The laboratory incubation experiment was carried out to investigate the feasibility of incorporating rye biomass harvested at different stages to mitigate CH₄ production in paddy soil. We found that controlling the harvest season of the cover crop can indeed be used to mitigate CH₄ emissions in rice soils treated with rye biomass as a green manure. To minimize the realistic CH₄ production potential and to maximize the possible nutrient productivity (mainly N) in field conditions, CH₄ production potential per N productivity was considered (Fig. 2). We found the lowest value at the early harvesting stage (191 - 198 DAS), which might be the best harvesting stage for rye biomass used in rice paddy soils. Conclusively, the regulation of harvest stage of the cover crop is a useful way to mitigate CH₄ emissions and increase nutrient production in temperate paddy soils treated with green manure. However, further field studies will be required to evaluate the realistic impacts of controlling harvesting season of green manure. Many factors, such as CH₄ production, oxidation and rice plant variation will affect the CH₄ dynamics in rice soils treated with green manures (Le Mer and Roger, 2001).

5. Conclusions

Biomass productivities of cover crop rye increased significantly as the plants matured, but biomass N productivities were maximized at the flowering stage (198 - 205 DAS). CH₄ productivity in soils treated with rye biomass decreased significantly as more mature rye were used, and the age at harvest had a strong positive correlation with labile organic C and N. However, total CH₄ production potential, which was estimated by multiplying rye biomass productivity by maximum CH₄ productivity to extrapolate realistic CH₄ production in rice fields, was mainly influenced by biomass productivity, and the highest potential was found at the maturing stage. As a result, CH₄ production potential per N productivity was lowest at the flowering stage, which could be the best harvesting stage to maximize biomass nutrient production

and minimize CH₄ emissions in rice paddies. Conclusively, the selection of harvest stage for cover crops can be used to increase nutrient production and decrease GHG emissions in arable lands.

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Table 1. Biomass production and chemical properties of rye biomass harvested at different growth stages, and correlations between total CH₄ production and plant chemical properties (n=21).

Parameters	Days after seeding (DAS)								HSD _{0.05}	Co-efficient (r)
	191	198	205	212	219	226	233			
Biomass productivity (Mg dry weight ha ⁻¹)	1.6	3.1	4.8	5.2	5.6	7.2	7.0	1.0	-	
Total organic C (g kg ⁻¹)	411	407	404	402	407	401	402	9	0.504 [*]	
Total N (g kg ⁻¹)	35.1	34.7	22.1	12.5	0.86	0.80	0.41	3.0	0.838 ^{***}	
C/N ratio	11.8	11.7	18.3	32.6	48.5	50.9	101.3	17.8	-0.726 ^{***}	
Total P ₂ O ₅ (g kg ⁻¹)	21.0	20.7	20.8	16.4	16.1	11.7	8.0	3.1	0.744 ^{***}	
Total K ₂ O (g kg ⁻¹)	4.9	5.1	5.7	4.0	2.5	1.9	2.1	1.5	0.700 ^{***}	
Water extractable C (g kg ⁻¹)	21.3	18.3	13.8	10.9	7.5	6.9	6.3	2.6	0.887 ^{***}	
Hot water extractable C (g kg ⁻¹)	26.7	23.1	16.8	14.2	9.7	8.5	7.8	3.4	0.885 ^{***}	
Water extractable N (g kg ⁻¹)	1.06	0.85	0.61	0.35	0.12	0.06	0.04	0.09	0.884 ^{***}	
Hot water extractable N (g kg ⁻¹)	1.36	1.14	0.72	0.53	0.18	0.10	0.06	0.12	0.882 ^{***}	
Cellulose (%)	20.0	22.2	24.0	24.4	26.7	27.5	27.6	4.2	-0.708 ^{***}	
Hemicellulose (%)	15.1	15.1	15.5	13.7	17.4	14.4	14.2	3.0	-0.161	
Lignin (%)	16.2	17.3	23.3	23.8	26.0	27.9	28.5	3.1	-0.827 ^{***}	
Total CH ₄ production (g kg ⁻¹ dry soil)	5.42	3.11	3.14	2.70	1.60	1.33	1.11	1.01	-	

Note) HSD_{0.05} indicates Tukey's post-hoc test at 5% level. ^{*}, ^{**} and ^{***} denote significance at 5%, 1% and 0.1% level, respectively.

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- Figure captions**

Figure 1. Changes in cumulative CH₄ production with rye incorporated at different harvesting stage. Bars represent standard deviations (*n*=3).

Figure 2. Estimated CH₄ production (A), N input (B) and CH₄ per unit N input (C). Bars represent standard deviations (*n*=3).

Figure 1.

Figure 2.