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<td>DOI</td>
<td>10.14943/doctoral.k12007</td>
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Effect of manure application on carbon budget in managed grassland and corn field in southern Hokkaido, Japan

Atfritedy Limin

PH.D. Dissertation

Laboratory of Soil Science
Division of Environment Resources
Graduate School of Agriculture, Hokkaido University
Sapporo, JAPAN
Acknowledgments

I’m grateful to everyone who helped me to complete this thesis:

To my supervisor, Professor of Soil Science, Ryusuke Hatano, for his kind and motivating supervision throughout the course of my study and invaluable comments and suggestions on the preparation of the manuscript;

To Professor Munehide Ishiguro and Professor Takashi Hirano for their comments and suggestions on the manuscript;

To Dr. Kanta Kuramochi, Shiori Asano, Yoshino Nakamura and Kazumi Ishikawa for their support and continuous help throughout my study and stay in Japan;

To Dr. Mariko Shimizu for supporting and helping me with the research;

To the kind friends and colleagues Fuyuko Hazama, Chihiro Naito, Kiwamu Ishikura, Kei Takizawa, Kajihara Naoya, Sachiko Takahashi, Hirono Kishimoto, Li Xi, Zamira, Li Mengjie, Chunying Wang, Fu Yang, Ikabongo Mukumbuta, Hitoshi Tsukasaki, Yuta Nibuoka, Hirasawa Shuntaro, Sho Saito, Shinya Iwasaki, Hirokazu Nakamoto, Yosuke Morimitsu, Arata Nagatake, Kentaro Okura, Kayoko Ogura, Kentaro Tanabe, Motohiro Yoshimura, Shunsuke Morita, Haiki Yupi, Fengky Adji, Rina Kartikawati, Priyo Nugroho and Vecky for their support and helping me with the field work, research and Japanese life experience;

I also thank the technical staff of Niikappu station and especially Professor Hiroshi Hata and Professor Seiji Kondo for their collaboration in the field investigation.

To MONBUKAGAKUSHO for the scholarship supporting my study in Japan.

To my family, Dr. Suwido Limin, Agustina Dewel, Dr. Sanz Grifrio Limin, and Zevy Augrind Limin for their support and motivation.
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Chapter 1. General Introduction

Recently, research in natural resources management, greenhouse gases emission and agro-ecosystem sustainability has attracted more and more attention. Carbon dioxide (CO₂) transfer in agricultural ecosystems is important not only for understanding greenhouse gases emission from the agricultural ecosystem but also provide the mechanism for photosynthesis, respiration and crop yield formation. These transfers determine whether an ecosystem is a source or sink of carbon (C). Between 1970 and 2004, global emissions of greenhouse gases increased by 70%, from close to 29–49 GtCO₂-eq, with CO₂ as the largest source, representing an increase of approximately 80% (Barkert et al., 2007). The rate of increase of atmospheric CO₂ concentration has been about 1.5 ppm (0.4%) per year over the past two decades (IPCC, 2001). Moreover, CO₂ is the most abundant trace gas and is radioactively active in the atmosphere responsible for 50% of the greenhouse warming effect (Bouwman, 1990). Therefore, quantifying the CO₂ exchange provides valuable knowledge into the dynamics of the long-term carbon ecosystem.

Fig. 1.1 Schematic diagram of C balance.
The C moves into and out of the terrestrial ecosystem through several processes. The balance of C can be calculated by the differences between the rates of C input and output. The vast majority of the input C into an ecosystem is through photosynthesis. The C is then partitioned among various plant parts, such as leaves, stems and roots. The next C transfers are from the decay of organic matter in litter and soil. C in the ecosystem is then released back into the atmosphere through respiration. These internal C processes are universal although their rates vary with ecosystems and environments. Fig. 1.1 shows the movement of C in terrestrial ecosystem in this study. The main C import into the ecosystem occurs via photosynthesis as assimilation of atmospheric CO₂ (gross primary production; GPP). This uptake is counterbalanced to a large part by respiration of plants and soil micro-organisms (ecosystem respiration; RE). The difference between these two processes (GPP - RE) is denoted as net ecosystem production (NEP). The NEP represents mainly the amount of C used to produce new biomass. Therefore, NEP is also described as the difference between net primary production (NPP) and soil heterotrophic respiration (RH). Management practices such as harvest and manure application can affect the C balance in the ecosystem. When aboveground biomass is harvested, the contained C is exported from the ecosystem. In the case of manure applications, C is imported into the ecosystem and has to be considered as well in the C budget. The sum of all C imports and exports equals the change of the C stored in the grassland soil (net biome production; NBP)

Grassland ecosystems, which cover approximately 40% of the earth's terrestrial area, are important ecological and socio-economic resources through their contribution to biodiversity and their use for feed, forage, and livestock production
Many studies have reported that grassland ecosystems function as potential C sinks or are near equilibrium with respect to C exchange. For example, a southern Great Plains mixed-grass prairie has been identified as carbon sink (Sims and Bradford, 2001); however a native tallgrass prairie in Texas (Dugas et al., 1999), non-grazed mixed-grass prairie in North Dakota (Frank and Dugas, 2001), and tallgrass in Oklahoma (Suyker et al., 2003) were found to be near equilibrium in terms of C. Although these studies suggested that grassland might be C sinks or near equilibrium, alternation between acting as C sink and C source frequently occurs. For example, a switch from sink to source was observed in a pasture in the southern Great Plains (Meyers, 2001), in a Canadian temperate mixed prairie during drought (Flanagan et al., 2002), and in a warm temperate grassland in southeastern U.S after harvesting (Novick et al., 2004).

Studies on C storage suggest that most of the C in grassland originates from belowground biomass (Hungate et al., 1997; Jackson et al., 2002). Grasslands have greater root biomass compared to annual crops, and grass roots play an important role in the C cycle and in C storage (Dupont et al., 2010). McNaughton et al. (1998) and Luo et al. (2009) measured the belowground net primary production (BNPP) by using root soil cores and estimation of BNPP from root biomass and root turnover rates and found that BNPP could represent more than half of the total net primary production (NPP), and represents the major input of organic matter into soil. However, knowledge of BNPP is limited compared to aboveground NPP (ANPP) due to the methodological difficulties associated with observing and measuring root biomass (Milchunas and Lauenroth, 2001; Wu et al., 2011). The NEP is defined as the difference between NPP and RH. In this study, NEP was provided by eddy covariance measurement directly, while ANPP and RH were provided by biometric measurement.
to assess the C budget and dynamics. Therefore, BNPP was estimated by using the combination of eddy covariance and biometric methods.

Land use change is regarded as the second largest anthropogenic source of greenhouse gas emission (IPCC, 2000). Most changes in land use affect the amount of C held in vegetation and soil by either releasing CO$_2$ to, or removing it from the atmosphere. Organic C storage in the soil is estimated to be greater than that in the atmosphere or biosphere (Batjes, 1996). Consequently, a small change in the soil C pool may affect the atmospheric CO$_2$ concentration (Wang et al., 1999). Soil surface CO$_2$ efflux is the main pathway of C emission from the soil to the atmosphere (Peng et al., 2008). Between 1750 and 2011, land use change activity (mainly from deforestation) has released 180 ± 80 Pg C to the atmosphere (Ciais et al., 2013). Several studies have reported that the land use change could increase the soil CO$_2$ fluxes of RS. Grover et al. (2012) measured the soil CO$_2$ flux of RS by using automated chambers and found that the conversion from savanna to pasture in north Australia increased the soil CO$_2$ flux of RS by 30%. However, land use change also can decrease or have no effect on soil CO$_2$ flux of RS. Sheng et al. (2010) measured soil CO$_2$ flux of RS using automated soil CO$_2$ flux system (LI-8100, Li-Cor Inc) in the subtropical region of China and found that the annual soil CO$_2$ flux of RS was reduced by 32% following the conversion of natural forest to secondary forest, 46-48% to plantations, 63% to citrus orchard and 50% to sloping tillage land, with the average reduction of 48%. Liu et al. (2011) measured soil CO$_2$ flux of RS using static closed chamber method in subtropical region of China and found that the conversion of the natural subtropical broadleaf evergreen forest to Moso bamboo does not increase soil CO$_2$ flux of RS.
Management practices and climatic conditions could affect the ecosystem’s capacity as either C source or sink (Peng et al., 2009; Sheng et al., 2009; Shimizu et al., 2009). Some management practices that increase the amount of organic C added to the soil such as continuous crop cover, elimination of fallow, increasing yield or manure application will potentially aid in sequestering atmospheric C (Janzen et al., 1998, Shimizu et al., 2009). Practices such as tillage and crop residue removal decrease the amount of organic C in the soil.

Several studies (Zenone et al., 2011; Zenone et al., 2013) have pointed out that the conversion of natural habitats to cropland would release CO₂. Zenone et al. (2013) found that the conversion from perennial grassland into no-till cropland induced a large C emission and caused the ecosystem to become a C source. However, the knowledge of effect conversion of perennial grassland into annual cropland with continuous manure application treatment on the C budget is limited, as manure application could enhance the C sequestration (Shimizu et al., 2009). In Shin-Hidaka city located in southern Hokkaido, large-scale arable land farming is practiced under a crop rotation system between grassland and corn field with manure application treatment.

The effect of land use conversion on the C budget with the manure application treatment is continuously measured without disturb the study area. Eddy covariance and chamber method are two main methods used for measuring ecosystem CO₂ fluxes. The main advantages of eddy covariance method are the absence soil disturbance during measurements and the ability to measure fluxes continuously for long period of time in relatively large area. Chamber method is used to measure the spatial and temporal change of CO₂ flux in a relatively small area. Its main advantages are the easy of use, low cost, and ability to measure the specific C
component. Therefore, in this study, the C budget was examined by using both eddy covariance method and chamber method.

The aim of this study was to understand the effect of management practices (i.e. manure application) on the different components of C cycle and their influence on the C budget in managed grassland and corn field. Thus, the objectives of this study are: (1) to determine a reasonable method to estimate BNPP, especially in managed grassland, (2) to clarify the effect of management practices such as harvest and continuous manure application on the C cycle in grassland, (3) to find out the effect of land use conversion from managed grassland to corn field on the various components [C input (manure application) and output (RH, RS, NPP, harvest)] of the C budget.

This dissertation is divided into six chapters. The current chapter (Chapter 1) is a general introduction to the study. Chapter 2 provides a review of the relevant literature on the research topic. Chapter 3 describes the materials and methods used during the research. Chapter 4 reports on the evaluation of manure application on the C balance of managed grassland. Chapter 5 mentions about the land use change effect on C balance from a managed grassland to corn field. Finally, a general discussion on the result of this study is presented in Chapter 6.
Chapter 2. Literature review

2.1 Carbon balance of an ecosystem

The C cycle is one of the most studied topics in the natural sciences (Fenn et al., 2015; Xu et al., 2014; Zanotelli et al., 2015). The increasing concentration of CO$_2$ in the atmosphere since the industrial revolution is the most significant human influence on the global environment (Malhi et al., 2002), driving much of the research on the global C cycle. The global C cycle involves the C cycling among the atmosphere, the oceans, and the vegetation and soils of the earth’s terrestrial ecosystems. Atmospheric CO$_2$ increased by 40% from 278 ppm in 1750 to 390.5 ppm in 2011 (Ciais et al., 2013) with anthropogenic CO$_2$ emissions to the atmosphere of about 555 ± 85 Pg C over the same period (IPCC, 2013; Ciais et al., 2013). Of this amount land use change (including deforestation, afforestation and reforestation) contributed 180 ± 80 Pg C. The increase in CO$_2$ emissions from fossil fuel burning and from land use change is the dominant cause of the observed increase in atmospheric CO$_2$ concentration (Ciais et al., 2013).

The C balance of an ecosystem at any point in time is the difference between its C gains and losses (Heimann and Reichstein, 2008). Carbon enters the ecosystem through photosynthesis, the processes by which autotrophs (plants and photosynthetic bacteria) produce their own organic compounds; most of this C returns to the atmosphere by autotrophic and heterotrophic (animals, fungi and micro-organisms) respiration (others losses of C could be as volatile organic compounds, methane or dissolved C).

The metabolic processes of organisms hence constitute the engine that drives the global C cycle on time scales of seconds (e.g., photosynthetic rates,) to centuries (large-scale climatic changes) (Chapin et al., 2011). The major C pools are: the
atmosphere, oceans, vegetation and soil, sediments and rocks. Within these, while the atmosphere is the smallest but most dynamic C pool, the terrestrial biosphere is the largest biological C reservoir (Chapin et al., 2011).

2.2 Environmental control on C balance

Components of the C balance such as photosynthesis and respiration often depend upon more than one factor. Net ecosystem exchange (NEE), the balance between photosynthetic uptake and release of CO₂ by ecosystem respiration from autotrophic and heterotrophic, represent the carbon sequestration between terrestrial ecosystem and the atmosphere during a given period. A number of environmental factors can play important roles in governing the rate of NEE. Several studies have shown that micrometeorology parameters such as air temperature, precipitation and photosynthetically active radiation (PAR) controlled the dynamics of NEE. Wang et al. (2008) studied the environmental effects on NEE for three years in a typical Stipa krylovii steppe in northern China using eddy covariance method and found that: (1) magnitude of NEE was strongly regulated by annual precipitation, (2) soil water content was important environmental factor regulating the variation of NEE, and (3) air temperature influenced the NEE-PAR relationship. The NEE between an ecosystem and the atmosphere is the net balance of GPP and RE. Chen et al. (2013) reviewed the environmental effects on the GPP and RE. The NEE of different terrestrial ecosystems in the Asian region was quantified based on long-term observation data of ChinaFlux (19 sites) and published data from AsiaFlux (37 sites) and 32 other sites in Asia. The studies found that the spatial variation of GPP and RE were mainly controlled by climatic factors such as mean annual temperature and mean
annual precipitation. The GPP is also affected by abiotic factors such as PAR, temperature and soil moisture (Lambers et al., 1998)

Soil respiration (RS) is the sum of respiration from ground vegetation, roots, rhizosphere, mycorhizia and microbes. There are many different factors that control RS, but temperature and moisture are the dominant factors (Lloyd and Taylor, 1994; Davidson et al., 2000; Swason and Flanagan, 2001). The temperature sensitivity (Q_{10}) of RS varies under different temperature ranges (Kirschbaum, 1995) and with the different RS components such as root and microbes (Boone et al., 1998; Janssens et al., 2003). Temperature and respiration from the different components fluctuate seasonally and the temperature sensitivity differs accordingly (Rayment and Jarvis, 2000; Widén, 2000).

2.3 Eddy covariance technique

Eddy covariance measurements were first made about 40 years ago, but became more widely used in the late 1980s (Baldocchi et al., 1988). More recently, eddy covariance technique is used to evaluate the exchange of C between the vegetation and atmosphere over a variety of ecosystems (Barr et al., 2007; Dunn et al., 2007).

Eddy covariance method provides a direct measurement of the net exchange of CO\textsubscript{2}, water vapor and sensible heat between a vegetated surface and the atmosphere (Baldocchi et al., 1988). The technique relies on atmospheric turbulence generated from the movement of air immediately above the earth’s surface. Atmospheric turbulence causes the formation of eddies which transport heat and mass between the surface and free atmosphere. The basic principle of this technique is that the vertical flux can be calculated as covariance between concentration of the entity of interest (e.g., CO\textsubscript{2}) and vertical wind speed in the eddies (Burba and Anderson, 2007).
Therefore, during periods of C uptake by the ecosystem, CO₂ rich air moves down into the ecosystem, while CO₂ poor air is correlated with upward vertical wind velocity. The covariance of the atmospheric CO₂ concentration and the vertical wind gives an estimate of the net amount of C absorbed or released by the ecosystem, over a given area at half-hourly timescales with minimal disturbance to the underlying vegetation, providing a reliable measure of NEE (Baldocchi et al., 1988). This technique provides accurate and continuous measurements of NEE at an ecosystem spatial scale. From these measurements of NEE, GPP and RE can be derived (Reichstein et al., 2005; Wohlfahrt et al., 2005a; Lasslop et al., 2010). This flux partitioning was classified in those that use only night time data for the estimation of RE and those that exploit day time data or both, day and night time data using light response curve. For the estimation of the temperature sensitivity, the RE is related to temperature using the exponential regression model (Lloyd and Taylor, 1994). Then for the day and night time data, RE and GPP are simultaneously modeled as parts of one model equation with state dependent parameters, where RE is y-intercept from light response curve of GPP (Falge et al., 2001).

In mathematical terms, eddy flux is computed as the covariance between the instantaneous deviation in vertical wind speed (w’) from its mean value (w) and the instantaneous deviation in gas mixing ratio (c’), from its mean value (c) and multiplied by mean air density; the vertical flux can be expressed by the following general equation (Baldocchi, 2003):

\[ F = \overline{\rho_a \ w' c'} \]

where overbar denotes temporal averaging (e.g. half-hour or hour), the prime denotes the deviation from the mean, \( \rho_a \), the air density, \( w \) and \( c \) vertical wind speed and gas concentration, respectively. This equation of flux is derived from vertical flux
equation by using Reynold decomposition (i.e. break equation into means and deviations). Air density fluctuations and divergence or convergence of turbulence flux are considered to be negligible (Burba and Anderson 2010). The example of eddy covariance at a single point was shown in Fig. 2.1.

Fig. 2.1 Example of eddy covariance at a single point (modified from Burba and Anderson, 2010). The amount of gas that observed by eddy covariance at single point can be determined by the difference in amount of molecules gas that moves upward (red color curve) and downward (blue color curve) at almost same time (time 1 ~ time 2) and with a certain wind speed (wind speed 1 and 2)

As a convention, when the net flux is towards the vegetation, indicating net uptake of CO\(_2\) by ecosystem, NEE has a negative sign, and when the net flux is away from the surface, indicating release of CO\(_2\) from ecosystem to the atmosphere, NEE has a positive sign.

The NEE can also be expressed as:

\[-NEE = GPP - RE;\] as eddy covariance system computes the net flux of CO\(_2\).
The other components of the carbon cycle such as GPP and RE could be estimated by using modeling techniques (Llyod and Taylor equation (RE); light response curve (GPP)).

Common eddy covariance instrumentation is composed of a 3-dimensional (3-D) sonic anemometer for the measurement of wind speed in the three components \((w, u, v)\) and an infrared gas analyzer (IRGA) to evaluate atmospheric mixing ratios of CO\(_2\) and H\(_2\)O (or another gas of interest). Instruments are mounted on a tower above the vegetation, at a height that depends on site characteristics (height of the vegetation, extent of the fetch, range of wind velocity and frequency response of the instruments). The turbulent up and down motions occur in part at very small temporal scales and in part at larger ones, for this reason eddy covariance requires sophisticated instrumentation to capture these fluctuations. Several instrument models to perform measurements with very high frequencies such as 10 or 20 Hz have been developed, and are employed worldwide. An example of eddy covariance typical configuration is presented in Fig. 2.2.

![Eddy Covariance Installation](image)

**Fig. 2.2** Example of an eddy covariance installation, composed by (A) a 3-D sonic anemometer (CSAT3, Campbell Scientific, Inc) and (B) open path IRGA (LI-7500, LI-COR, Inc., Lincoln, NE, USA).
Two different functional modalities exist for IRGA: closed-path system and open-path system. In closed-path systems air is actively pumped to the sample cell through a tube, while open-path gas analyzer assesses “in situ” free air. Both have advantages and disadvantages, and are generally more or less suitable to different environmental conditions. Open-path sensors require low power supply, don’t have pump and tube and are suitable for harsh environments, on the other hand, they suffer of loss of data when some environmental conditions such as precipitation and snow cover or make dirty the sensor window. Open-path sensors do not directly measure CO\textsubscript{2} and H\textsubscript{2}O mixing ratios but their densities. As atmospheric gas densities could be influenced by fluctuations in pressure, temperature and humidity that generate expansion and contraction of air volume, corrections (Webb et al. 1980, WPL) to compensate these effect on measured fluxes are required. Closed-path analyzers can output directly gas mixing ratios, making density corrections (WPL) less important, they don’t suffer of data loss due to precipitation and icing but have a high power requirement and are affected by CO\textsubscript{2}/H\textsubscript{2}O signal tubing attenuation.

Removing bad data is an important part of the data quality control processes. It ensures that results do not have a bias or errors due to some obvious or common reasons. Bad data are usually removed for one of the following causes: instrument malfunctions, processing or mathematical artifacts, ambient condition not satisfying eddy covariance method, winds are not from the footprint of interest, and heavy precipitation (Burba and Anderson 2010).
2.4 Effect of land use change on carbon balance

Land use and land-use change directly affect the exchange of greenhouse gases between terrestrial ecosystems and the atmosphere (IPCC, 2000). Changes such as the clearing of forests for use in agriculture or as settlements are associated with clear changes in land cover and carbon stocks. Much of the world's land area continues to be managed for food and wood production, human habitation, recreation, and ecosystem preservation without a change in land use (Briassoulis, 2000). Management of these land uses affects sources and sinks of greenhouse gases (IPCC, 2000). Furthermore, the resulting agricultural and wood products contain carbon. The carbon stocks held in these products are eventually released back to the atmosphere, after the products have served their use. Biomass carbon stocks are also used to produce energy that serves as a substitute for, and as complement to, fossil fuels.

Different factors and mechanisms drive land use and land cover transformation. In many cases, local climate and weather, topography, technology, and economics appear to be determinants of land-use change at different spatial and temporal scales (Briassoulis, 2000). Land-use change is often associated with a change in land cover and an associated change in carbon stocks (Guo and Gifford, 2002; Houghton, 2004). Houghton (1991) assessed seven types of land-use change for carbon stock changes: (1) conversion of natural ecosystems to permanent croplands, (2) conversion of natural ecosystems for shifting of cultivation, (3) conversion of natural ecosystems to pasture, (4) abandonment of croplands, (5) abandonment of pastures, (6) harvest of timber, and (7) establishment of tree plantations.

Studies on grassland CO$_2$ exchange have shown that they may act as either a source or sink of CO$_2$ (Leahy, 2004). Novick et al. (2004) collected information on annual grassland NEE estimates based on eddy-covariance measurements and Bowen
Ratio Energy Balance techniques and reported values varying from a net source of 400 g C m$^{-2}$ to a net sink of -88 g C m$^{-2}$. A review of available data has shown that large uncertainties remain in resolving whether grassland ecosystems function as CO$_2$ sources or sinks (Janssens et al., 2003). This uncertainty is primarily attributable to the sensitivity of grasslands to inter-annual variability in climate and associated biomass dynamics (Meyers, 2001; Flanagan et al., 2002) and incomplete understanding of the regulation of grassland assimilation and respiration (Wever et al., 2002).

Land-use change has been estimated to be a major global carbon source, adding about 124 PgC yr$^{-1}$ to the atmosphere, with the majority being lost due to conversion of forestry to agriculture as well as changes from grasslands to croplands (Houghton, 1999; Smith, 2008). Alberti et al (2010), by using eddy covariance and automated chamber, investigated short-term effects (2 years) of land conversion from continuous maize (Zea mays L.) to alfalfa (Medicago sativa L.) on C balance and reported that NEP increased two years after conversion from corn to alfalfa (281 g C m$^{-2}$ yr$^{-1}$). However, continuous maize was a lower source of C in terms of NBP (-96 g C m$^{-2}$ yr$^{-1}$) than maize-alfalfa land use conversion (-354 g C m$^{-2}$ yr$^{-1}$). From the result, it appears that this type of land conversion is not an effective measure of C sequestration in the short term (1–3 years), but it could be in the long term.

Zenone et al (2011) studied the effects of land use change from perennial grassland to an annual crop (soybean) in southwestern Michigan, US. The study examined the effect of land conversion on carbon (C) fluxes using the eddy covariance technique at seven sites and quantified the effect of major management activities on C fluxes during the conversion year. Four sites had been managed as grasslands under the Conservation Reserve Program of the USDA. Three fields had
previously been cultivated in a corn/soybean rotation with corn until the year before this study. The effects of land use change were studied during 2009 when six of the seven sites were converted to soybean cultivation, with the seventh site kept as grassland. In winter (December to March), the corn fields were C neutral while the grasslands were C sources, with average emissions of 15 g C m$^{-2}$ month$^{-1}$. In April, first year of conversion, while the corn fields continued to be a C source to the atmosphere, the grasslands switched to C sinks. The conversion of grassland to soybean induced net C emissions with NEE ranging from 155.7 ± 25 to 128.1 ± 27 g C m$^{-2}$ yr$^{-1}$. The annual NEE at the unchanged plot (grassland) was -81.6 ± 26.5 g C m$^{-2}$ yr$^{-1}$ while at the plot converted from corn/soybean rotation was remarkably different with two sites being sinks of -91 ± 26 and -56.0 ± 20.7 g m$^{-2}$ yr$^{-1}$ whereas one site was a source of 31.0 ± 10.2 g C m$^{-2}$ yr$^{-1}$. This study concluded that the conversion of perennial grasslands back to an annual cropping system induced a large emission of CO$_2$ during the first year of cultivation, mainly due to the dead aboveground biomass present after the herbicide application and decomposition of roots. On the other hand, the undisturbed reference grassland continued to be a C sink.

2.5 Effect of management practices

Management of croplands, and grassland affects sources and sinks of greenhouse gases (CO$_2$, CH$_4$, and N$_2$O) (Brandyopadhyay and Lal, 2014; Linguist et al., 2012; Shimizu et al., 2009). Cropland soils can lose C as a consequence of soil disturbance (e.g., tillage). Tillage increases aeration and soil temperatures (Tisdall and Oades, 1982; Elliott, 1986), making soil aggregates more susceptible to breakdown and physically protected organic material more available for decomposition (Elliott, 1986; Beare et al., 1994). In addition, erosion can significantly affect soil C stocks
through the removal or deposition of soil particles and associated organic matter. Erosion and redistribution of soil may not result in a net loss of carbon at the landscape level because carbon may be re-deposited on the landscape instead of being released to the atmosphere (van Noordwijk et al., 1997; Lal et al., 1998; Stallard, 1998). Although some organic matter may be re-deposited, the productivity of the eroded soil is decreases due to the reduced ability to support C fixation and storage. Losses through leaching of soluble organic C occur in many soils; although this leaching is seldom a dominant C flux in soils, it is a contributor to the transport of C from the terrestrial environment to the marine environment via runoff (Meybeck, 1982; Sarmiento and Sundquist, 1992). Soil C content can be protected and even increased through alteration of tillage practices, crop rotations, residue management, reduction of soil erosion, improvement of irrigation and nutrient management, and other changes in forestland and cropland management (Kern and Johnson, 1993; Cole et al., 1996).

Livestock grazing on grasslands, converted cropland, savannas, and permanent pastures is the largest areal extent of land use (FAO, 1993). Grazing alters ground cover and can leads to soil compaction and erosion, as well as alteration of nutrient cycles and runoff. Soil C, in turn, is affected by these changes. Avoiding overgrazing can reduce these effects (Mscherry and Ritchie, 2013). Ecosystem conservation may also influence carbon sinks. Many forests, savannas, and wetlands, if managed as natural reserves or/recreation areas, can preserve significant stocks of C (IPCC, 2000), although these stocks might be affected negatively by climate change (IPCC, 2000). Some wetlands and old-growth forests exhibit particularly high C densities (Erwin, 2009; Marton et al., 2014) while other semi-natural ecosystems (e.g.,
savannas) may conserve C simply because of their large areal extent (Alam et al., 2013).
Chapter 3. Materials and methods

3.1 Site description and treatments

This study was conducted from 2008 to 2014. The study site is located at Niikapu station, National Livestock Breeding Center, Shin-Hidaka city, Southern Hokkaido (42°24 N, 142°28 E). This region belongs to the cool-temperate zone and is characterized by cold winters and warm summers without apparent wet or dry season. The mean annual precipitation is 1365 mm and the mean annual temperature is 7.9°C, with the mean monthly temperature ranging from 20.6°C in August to -5.7°C in January (Shimizu et al., 2010). The site is covered with snow from the end of December to the beginning of March.

The grassland was established more than 100 years ago, and renovated in 2004 from grazed grassland. Dominant grass species in this site were orchard grass \((Dactylis glomerata\ L.\) and meadow fescue \((Festuca pratensis\ Huds.)\). In late August of 2010, over-seeding of orchard grass \((10\ \text{kg}\cdot\text{ha}^{-1})\) and red clover \((5\ \text{kg}\cdot\text{ha}^{-1})\) was conducted at the study site, by using a rotary-tilling-type grassland renovator with narrow tilling device, to maintain grass productivity. Herbicide was applied to the field in mid-October 2012 to convert the grassland to corn field in 2013.

The soil is derived from Tarumae-b volcanic ash (Jin et al., 2010; Shimizu et al., 2010), and is classified as Andosol (IUSS Working Group WRB, 2006). The soil profile survey was conducted in September 2007 before the treatment. The soil physical and chemical properties are shown in Table 3.1. The thickness of the Ap-horizon was 26 cm and total C and total N contents of the Ap-horizon were 86 and 6.3 g·kg\(^{-1}\), respectively with a dry bulk density of 0.63 Mg·m\(^{-3}\).
Table 3.1 Detailed description of the soil characteristics.

<table>
<thead>
<tr>
<th>Horizon</th>
<th>Depth (cm)</th>
<th>pH</th>
<th>Dry bulk density (Mg·m⁻³)</th>
<th>Total C (g·kg⁻¹)</th>
<th>Total N (g·kg⁻¹)</th>
<th>Sand (g·g⁻¹)</th>
<th>Silt (g·g⁻¹)</th>
<th>Clay (g·g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ap1</td>
<td>0-6</td>
<td>5.7</td>
<td>0.620</td>
<td>85.4</td>
<td>5.8</td>
<td>0.70</td>
<td>0.21</td>
<td>0.09</td>
</tr>
<tr>
<td>Ap2</td>
<td>6-26</td>
<td>5.4</td>
<td>0.639</td>
<td>87.3</td>
<td>6.6</td>
<td>0.71</td>
<td>0.21</td>
<td>0.08</td>
</tr>
<tr>
<td>2A</td>
<td>26-40</td>
<td>5.6</td>
<td>0.458</td>
<td>180.5</td>
<td>10.2</td>
<td>0.49</td>
<td>0.32</td>
<td>0.19</td>
</tr>
<tr>
<td>3A</td>
<td>40-55</td>
<td>5.5</td>
<td>0.491</td>
<td>107.2</td>
<td>6.7</td>
<td>0.44</td>
<td>0.37</td>
<td>0.18</td>
</tr>
<tr>
<td>3C</td>
<td>55-100+</td>
<td>5.6</td>
<td>0.538</td>
<td>35.0</td>
<td>2.5</td>
<td>0.56</td>
<td>0.32</td>
<td>0.12</td>
</tr>
</tbody>
</table>

The survey was conducted in September 2007 before the treatment.

3.2 Management practices

3.2.1 Managed grassland

Two adjacent experimental plots (170 m × 150 m) were set up; in one plot only chemical fertilizer was applied (fertilizer plot; F plot) and the other plot was applied with manure and supplementary chemical fertilizer (manure plot; M plot). In order to measure the soil organic matter decomposition and manure decomposition, small chamber plots were set up (10 m × 10 m) (Fig. 3.1).
Fig. 3.1 The layout of the experimental plots.
Table 3.2 The date of chemical fertilizer and manure applications, and the amount applied in the fertilizer (F) and manure (M) plots.

<table>
<thead>
<tr>
<th>Year</th>
<th>Date</th>
<th>Application rates (kg·ha⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F plot</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chemical fertilizer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T-N</td>
</tr>
<tr>
<td>2008</td>
<td>Apr. 30</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Jun. 17</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Aug. 22</td>
<td>20</td>
</tr>
<tr>
<td>2009</td>
<td>Apr. 24</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Jun. 15</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Aug. 11</td>
<td>30</td>
</tr>
<tr>
<td>2010</td>
<td>Apr. 26</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>Jun. 18</td>
<td>22</td>
</tr>
<tr>
<td>2011</td>
<td>May 5</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Jun. 21</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Aug. 22</td>
<td>19</td>
</tr>
<tr>
<td>2012</td>
<td>May 14</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>Jun. 14</td>
<td>17</td>
</tr>
<tr>
<td>2013</td>
<td>Jan. 21</td>
<td>150</td>
</tr>
<tr>
<td>2013</td>
<td>Oct. 16</td>
<td>136</td>
</tr>
</tbody>
</table>
The harvesting of grass was carried out two or three times per year. In 2010, the 3rd crop was cut but was not removed from the field because of low volume of production. The 3rd crop of 2012 was also not harvested due to the herbicide application for renovation. The information on the date of application and the application rates of fertilizer and manure are given in Table 3.2. The fertilizer application rates in the F plot ranged from 50 to 100 kg N ha$^{-1}\cdot$yr$^{-1}$. The amount of manure applied to the M plot was 10 Mg fresh matter ha$^{-1}\cdot$yr$^{-1}$ (1923 ± 407 kg C ha$^{-1}\cdot$yr$^{-1}$, 159 ± 68 kg N ha$^{-1}\cdot$yr$^{-1}$). In the M plot, the nutrient supply rate from manure was estimated by multiplying the application rate by the mineralization rate, and the difference between the supply rate from manure and the application rate in F plot was supplied with chemical fertilizer. The N mineralization rate was estimated based on Uchida’s model, which was developed in Japan (Shiga et al., 1985). According to this model, the cumulative N mineralization rate (1 – $y_t$) was estimated to be 0.132 in the applied year ($t = 1$), 0.202 in two years ($t = 2$), and 0.257 in three years ($t = 3$), respectively. The equation is as follows:

$$y_t = a \times 0.01t + c \times 0.63t + f \times 0.955t$$  \[3.1\]

where $y_t$ is the rate of N remaining in the manure $t$ years after application, $t$ is the years after manure application (inclusive of the applied year), $a$, $c$, and $f$ are the rates of organic matter fractions with different decomposition rates ($a + c + f = 1$). In the case of cattle manure, $a$, $c$, and $f$ were estimated to be 0.04, 0.15, and 0.81, respectively. Therefore, the annual N mineralization rates were 13.2, 7.0, 5.5, 4.6, and 3.9% of the total manure N in the first to fifth years after manure application, respectively. The rate of phosphorus and potassium mineralization from the manure was estimated based on a handbook on animal waste management and utilization in
Hokkaido published in 2004 (Hokkaido Prefectural Experiment Stations and Hokkaido Animal Research Center, 2004), namely 20% of phosphorus and 70% of potassium are available in the applied year, 10% of phosphorus and 10% of potassium are available the second year, respectively.

We defined the crop-growing season as a 7-day moving average of daily air temperature above 5 °C and the non-growing (NG) season as the rest. The length of the growing season ranged from 202 to 228 days. The growing season was divided into three or four periods; from the beginning of the growing season to the first crop harvest (G1), from the first harvest to the second harvest (G2), from the second harvest to the third harvest (G3), and from the third harvest to the end of the growing season (G4) (Table 3.2). In 2010 and 2011, the 3rd crop was cut towards the end of the growing season, and was not calculated for the G4 period. We did not estimate the G4 period in 2010, 2011, and 2012 due to the late cutting or herbicide use in those years.

3.2.2 Corn field

Corn field was established from 2013 to 2014. There was only one experimental plot; applied by manure and supplementary chemical fertilizer. The study site was ploughed in the end of April. In the beginning of May, corn was planted and was harvested at the end of October. The amount of manure applied in the corn field was 50 Mg Fresh matter ha⁻¹·yr⁻¹ (4864 ± 823 kg C ha⁻¹·yr⁻¹, 268 ± 108 kg N ha⁻¹·yr⁻¹). The nutrient supply rate from manure and N mineralization rate was estimated the same way as mentioned previously for the managed grassland.
3.3 Eddy covariance system and meteorological station

All measurements were performed according to those carried out in a previous study conducted at Shizunai experimental farm (Hirata et al., 2013). An eddy covariance system was set up in both F and M plots in the managed grassland and M plot in the corn field to measure net ecosystem exchange (NEE) of CO₂. We employed an open-path eddy covariance system, which consisted of a three dimensional sonic anemometer (CSAT3; Campbell Scientific, Logan, UT, USA), open-path infrared gas analyzer (IRGA) (LI-7500; Li-Cor, Lincoln, NE, USA), and a data logger (CR23X, Campbell Scientific) equipped with a compact flash recorder. The sensor head of the sonic anemometer and IRGA were set at 2.45 m and 5.4 m above the ground for managed grassland and corn field, respectively. The IRGA was calibrated in the laboratory at least once a year using dry zero gas (pure air, CO₂ < 0.1 ppm), CO₂ span gas (500 ppm CO₂ in air), and H₂O span gas supplied from a dew-point generator (LI-610, Li-Cor).

Air temperature and relative humidity were measured at the same height that the flux measurement was taken using a temperature-humidity sensor (HMP-45A, Vaisala, Helsinki, Finland) equipped with a homemade ventilator. Incoming photosynthetically active radiation (PAR) was measured above the soil surface (1.6 m in managed grassland and 5.4 m in corn field), using a quantum sensor (LI-190SL, Li-Cor). All meteorological measurements were sampled at 1 s intervals and averaged over 30 min. Gaps in the meteorological data due to power failure were filled with data from the nearest Automated Meteorological Data Acquisition System (AMeDAS) station of the Japan Meteorological Agency. Precipitation data were obtained from the nearest AMeDAS station, which is located about 20 km from the study site.
Half-hourly CO$_2$ fluxes were calculated from covariance between vertical wind speed and CO$_2$ density. We used standard procedures (Mano et al., 2007) to correct the coordinate rotation of the wind field, the influence of water vapor flux on sonic thermometry, high frequency of cospectral losses due to sensor separation and path-length averaging, and the influence of air density fluctuations due to variation of air temperature and water vapor. Non-stationarity and integral turbulence tests (Foken and Wichura, 1996) were applied to discard any erroneous half-hourly flux data obtained under unfavorable atmospheric conditions or by malfunctioning instruments. Because the measurement height was 2.5 m in managed grassland and 5.4 m in corn field, the changes in CO$_2$ storage below this height were neglected, and the CO$_2$ flux was therefore regarded as NEE. Fluxes were also discarded for periods when friction velocity ($u^*$) was below the critical threshold of 0.05 m·s$^{-1}$ (Hirata et al., 2013). In total, data gaps caused by sensor malfunctioning, exclusion of data from the afforested sector, and data filtering during post-processing steps required gap-filling of 32 ± 2 and 34 ± 5% of the dataset during the observation period for F and M plots, respectively. In corn field, total of 45 ± 10% of data was gap-filled. NEP has the same magnitude as NEE but is represented by the opposite mathematical sign, i.e., positive NEP represents C uptake by the ecosystem.

We used the following equation according to Lloyd and Taylor (1994) to fill gaps in the nighttime NEE ($F_{\text{NEE,night}}$):

$$
F_{\text{NEE,night}} = F_{\text{RE,Tref}} \exp \left( \frac{E_0}{R} \left( \frac{1}{T_K + T_{\text{ref}}} - \frac{1}{T_K + T_a - T_0} \right) \right)
$$

[3.2]

where $F_{\text{RE,Tref}}$ is the $F_{\text{NEE,night}}$ at the reference temperature ($T_{\text{ref}}$), which was fixed at 10°C, $E_0$ is the activation energy (J·mol$^{-1}$), $R$ is the ideal gas constant (8.31 J·mol$^{-1}$·K$^{-1}$) and $T_a$ is the air temperature (°C). The values of $T_K$ and $T_0$ are 273.15 and 227.13 K,
respectively. The fitted parameters, $E_0$ and $F_{RE,\text{Ref}}$ were determined each day for a 15-day moving window by means of the least-squares method.

Data gaps in daytime NEE ($F_{\text{NEE,day}}$) were filled by the following non-rectangular hyperbola equation as a function of PAR (Q) (Prioul and Chartier, 1977).

$$F_{\text{NEE,day}} = \frac{\phi Q - P_{\text{max}} + \sqrt{(\phi Q + P_{\text{max}})^2 - 4\phi Q \theta P_{\text{max}}}}{2\theta} + R_d$$  \hspace{1cm} [3.3]

In this equation, $P_{\text{max}}$, $\phi$, $\theta$ (= 0.9) and $R_d$ are the maximum GPP at light saturation ($\mu$mol·m$^{-2}$·s$^{-1}$), the initial slope (mol·mol$^{-1}$), the convexity of the light response curve and daytime respiration ($\mu$mol·m$^{-2}$·s$^{-1}$), respectively. The fitted parameters, $P_{\text{max}}$, $\phi$ and $R_d$ were determined each day for a 7-day moving window by means of the least-squares method.

NEP is the difference between photosynthetic CO$_2$ assimilation by plants (gross primary productivity; GPP) and respiratory CO$_2$ released from plants and soil (ecosystem respiration; RE). We divided NEE into GPP and RE using a nonlinear empirical model. Nighttime RE equals nighttime NEE, because nighttime GPP is regarded as zero. Daytime RE was estimated from air temperature by extrapolation using Eq. 3. Daytime GPP was estimated by subtracting the observed NEE from daytime RE.

3.4 Measurement of soil respiration (RS) and heterotrophic respiration (RH)

Soil respiration (RS) was estimated as the soil surface CO$_2$ flux, which includes respiration of plant roots, rhizosphere, microbes and fauna, while RH in this study was estimated as the soil surface CO$_2$ fluxes from the root exclusion plot (Shimizu et al., 2009). In managed grassland, four root exclusion plots (1 m × 1 m) were established only in 2011 and 2012 in the chamber area (Fig. 3.1). In the root exclusion
plots in managed grassland, chemical fertilizer was applied at the same application rate and timing as in F plot, and manure was not applied. The soil surface CO$_2$ flux from the root exclusion plot with chemical fertilizer treatment is denoted as RHzs. In corn field, the root exclusion plots (1 m × 1 m) were established in 2013 and 2014 in the chamber area (Fig. 3.1). In the root exclusion plots in corn field, manure was applied at the same amount and timing as M plot. In order to obtain heterotrophic respiration of manure in corn field (RHm), root exclusion plots with manure application (RH) and with only fertilizer application (RHzs) were installed in the chamber area and RHm was obtained from the difference between RH and RHzs. In root exclusion plot, the aboveground plants and roots were removed with a shovel and by hand, and a root-proofing permeable sheet (BKS9812; TOYOBO, Osaka, Japan) was vertically inserted to a depth of 20 cm below the ground surface to inhibit regrowth of roots. The surface of the root exclusion plots was covered by a 1-mm-mesh nylon net to prevent the soil surface from aggregate degradation by rain, because this may enhance organic matter decomposition (Lundquist et al., 1999; Casals et al., 2009). The net could also prevent an increase of daily maximum soil temperature in the root exclusion plot.

Soil respiration and RH were measured using the static closed chamber method (Shimizu et al., 2009) (Fig. 3.2). The flux measurements were conducted bi-weekly during the crop-growing season, but the frequency was increased to twice per week after fertilization. The measurements were conducted monthly during the non-growing season. In managed grassland, two types of chamber were used. Small chambers which were made from stainless steel chambers (20 cm diameter and 25 cm high) were used to measure RH and big chambers (40 cm diameter and 30 cm high) were used to measure RS. In corn field, RH and RS was measured using the small
chambers only. The small chambers were placed on chamber-bases made of stainless steel with a diameter of 20 cm, which were kept on the ground except during harvesting. The big chamber was installed at a depth of 3 cm into the soil one day before the measurement day. The headspace gas samples from each chamber were collected and placed into a Tedlar bag before the chamber was closed and after 6 min under a closed-chamber condition. The CO$_2$ gas concentrations were determined in the laboratory using a CO$_2$ infrared gas analyzer (ZFP9GC11, Fuji Electric, Tokyo, Japan). Soil respiration and heterotrophic respiration were calculated using a two-point regression of CO$_2$ concentration in the chamber (Nakano et al., 2004).

![Diagram](Fig. 3.2 Outline of the chamber and base used in the closed chamber method (Jin, 2010; Toma and Hatano, 2007).)

Gas fluxes were calculated from the change in gases concentration in the chamber using following equation

$$F = \rho \times h \times (\Delta c/\Delta t) \times \left[\frac{273}{(273+T)}\right]$$

where $F$ is the gas flux (mg C m$^{-2}$ h$^{-1}$), $\rho$ is the gas density (CO$_2$-C = $0.538 \times 10^6$ mg m$^{-3}$), $h$ is the height of the chamber from soil surface (m), $\Delta c/\Delta t$ is the change in gas concentration inside the chamber during the sampling period (m$^3$ m$^{-3}$ h$^{-1}$), $T$ is the air
temperature inside the chamber (°C). A positive flux denotes the emission from the soil, whereas a negative flux denotes the uptake from the atmosphere.

Air and soil temperature were measured concurrently at depths of 5 cm with the CO₂ flux measurements using a thermistor thermometer (CT220, CUSTOM, Tokyo, Japan). Soil temperatures at 5 cm depth were also monitored every 30 min with a thermocouple thermometer (TR-52, T&D, Nagano, Japan). Relationship between the soil surface CO₂ flux from the root exclusion plot (Grassland: F plot; Corn field: F and M plots) and soil temperature at 5 cm depth was fitted by Lloyd and Taylor equation (Eq. 3.2). The RHs in managed grassland (F and M plots) and cornfield (F plot) and RH in M plot in corn field were estimated using the Lloyd and Taylor equation driven by the 30-minute mean soil temperature in the F and M plots. The RH in M plot in managed grassland was estimated by the sum of RHs in M plot and heterotrophic respiration of manure (RHm).

\[ Q_{10} = \exp \left\{ \frac{E_a}{R} \left( \frac{1}{T_k + T_{ref} - T_0} - \frac{1}{T_k + T_a - T_0} \right) \right\} \]  
\[ \text{[3.5]} \]

In managed grassland, the RH of manure (RHm) was estimated as the difference of RE between M and F plots as follows:

\[ \text{RHm} = \text{RE}_{\text{M plot}} - \text{RE}_{\text{F plot}} \]  
\[ \text{[3.6]} \]

And heterotrophic respiration in M plot of grassland was estimated as,

\[ \text{RH} = \text{RHs} + \text{RHm} \]  
\[ \text{[3.7]} \]

In F plot of managed grassland RHm is zero, heterotrophic respiration is written as:

\[ \text{RH} = \text{RHs} \]  
\[ \text{[3.8]} \]

In corn field, RHm was obtained from RH in M plot and RHs in F plot as follows:
RHm = RH – RHs \ [3.9]

Ecosystem respiration can be partitioned into autotrophic respiration (RA) and RH contributions. Autotrophic respiration was calculated as the difference between RE and RH

RA = RE – RH \ [3.10]

Autotrophic respiration can be further subdivided into aboveground autotrophic respiration and belowground autotrophic respiration (root respiration: RR). Root respiration is part of RS and is estimated as;

RR = RS – RH \ [3.11]

### 3.5 Plant biomass measurement

We divided plant biomass into two parts as the above ground biomass and the below ground biomass. In managed grassland, aboveground biomass was measured four or five times a year: in April, June (before 1\textsuperscript{st} crop harvest), August (before 2\textsuperscript{nd} crop harvest), October (before 3\textsuperscript{rd} crop harvest), and at the end of the growing season. The aboveground biomass at the time of harvest was estimated as the sum of the harvests and the stubbles and leaf litters. The harvest was measured by taking clippings 10 cm above the ground using the 100 cm \times 100 cm quadrants, and stubble biomass and leaf litters at the time of crop harvest and the aboveground biomass in April and the end of the growing season were measured by clipping using the 50 cm \times 50 cm quadrants with eight replications. The samples were oven-dried at 70°C for 72 hours and then weighed. The belowground biomass was measured by taking four soil blocks (50 cm \times 25 cm \times 30 cm deep) with four replications. The belowground biomass was collected four times a year: in April and at the end of G1, G2 and G3 periods. From 2008 to 2011, the belowground biomass in M plot was collected only at
the end of G3. In 2012, the belowground biomass was collected three times (in April, at the end of G1 and G2 periods) in both plots. The belowground samples were washed using a 0.5 mm sieve, over-dried at 70°C for 72 hours, and weighed. In corn field, aboveground biomass was measured at harvesting time (end of October) and divided into two parts as the harvest and residues (i.e. root and stubble). Eight plant samples were taken and dried at 70°C for 72 h and then weighed. Each dried sample of managed grassland and corn field was analyzed for total C and N contents with an N/C analyzer (SUMIGRAPH NC–1000, Sumika Chemical Analysis Service, Ltd., Osaka, Japan).

The ANPP was estimated as an increment in biomass during each period in the growing season (Shimizu et al., 2009). The annual ANPP in managed grassland was estimated by the sum of biomass increase in each period, while for corn field it was estimated from the harvested biomass. BNPP_{est} was estimated using the following equations,

\[ \text{BNPP}_{\text{est}} = \text{NEP} + \text{RH} - \text{ANPP} \] [3.12]

where NEP is provided by eddy covariance measurement, ANPP, RHs and RHm were provided by biometric measurement. NPP_{est} was calculated as the sum of ANPP and BNPP_{est}. BNPP from harvest method (BNPP_{bio}) was estimated from the root biomass. NPP was calculated as the sum of ANPP and BNPP_{bio}.

3.6 Soil sampling

Soil core samples were collected from four depths (0-5, 5-10, 10-20 and 20-30 cm) at 10 and 20 m distance from the EC tower in four directions from the tower (south, west, north and east). Total C content of soil was analyzed with an N/C
3.7 Net biome production

The annual net biome production (NBP) is net gain or loss of carbon in the field, which approximates change in soil organic C (Shimizu et al., 2009), and was calculated as following equation,

\[
NBP = NEP + \text{Manure application} - \text{Harvest} \quad [3.13]
\]

3.8 Statistical analysis

Statistical analyses were performed with SPSS Statistic (version 20; IBM Corp, Armonk, NY, USA) and R software (R Development Core Team, 2012; Ver. 2.15.1). The various components of the seasonal C budget among the treatments and seasons were compared using two-way repeated measures analysis of variance (ANOVA) and one-way repeated measures ANOVA for annual data. The differences between treatments and seasons were analyzed using the Tukey–Kramer (honest significant difference; HSD) test. The relationship between the C budget and each environmental variable was subjected to stepwise multiple regression analysis.

The two-sided 95% confidence interval of ANPP and BNPP were calculated (Shimizu et al., 2009), and uncertainties were calculated using the following equation:

\[
uncertainty = \frac{(\text{two-sided 95% confidence interval})}{\text{means}} \times 100 \quad [3.14]
\]

The uncertainties of annual ANPP and BNPP were estimated as the sum of uncertainties in ANPP and BNPP for each period using an error propagation.
Chapter 4. Manure application has an effect on the carbon budget of a managed grassland in southern Hokkaido, Japan.

4.1 Introduction

The use of manure is expected not only to reduce the dependence on chemical fertilizer but also to sequester the applied manure C in soils (Janzen et al., 1999). Cattle manure application on dry land and irrigated soils over 25 years in Canada increased the soil organic C through the addition of organic C in the manure (Whalen and Chang, 2002). Organic C that remains after application of manure is assumed to be a part of the soil organic C, and will be decomposed gradually over time, releasing plant nutrients in a way that resembles a slow release fertilizer (Brandjes et al., 1996). Understanding how the C dynamics are affected by long-term manure application is imperative to evaluate the effect of manure application on C sequestration and grass production.

In grassland, belowground net primary production (BNPP) could represent more than half of the total net primary production (NPP), and represents the major input of organic matter into soil (Briggs and Knapp, 1995; McNaughton et al., 1998; Luo et al., 2009). However, knowledge of BNPP remains limited in comparison to aboveground NPP (ANPP), which is better understood, largely due to the methodological difficulties associated with observing and measuring root biomass. Therefore, by using the combination of eddy covariance and biometric method we have developed a reasonable method to estimate BNPP in grassland.

Understanding BNPP dynamics is fundamentally important to improve our knowledge of C allocation and storage in grasslands. In this study, we have developed
a method to estimate BNPP by the combination of the eddy covariance and biometric methods. NEP is defined as the difference between NPP and heterotrophic respiration (RH).

The NEP of intensively managed grassland showed a net C sink (Gilmanov et al., 2010). However, these apparent sinks accumulate in crops and forage, which are C pools that are harvested, transported, and decomposed off site. Therefore, the C budget in managed grasslands includes C output through crop harvest and grazing and C input through manure application as well as NEP. This budget is defined as the net biome production (NBP) (Schulze et al., 2000)

The objectives of this Chapter were to determine a reasonable method to estimate BNPP in grassland and to clarify the effect of management practices such as harvest and continuous manure application on the C cycle in grassland.

4.2 Results

4.2.1 Environmental variables

The daily precipitation is shown in Fig. 4.1, and the cumulative precipitation for each period is shown in Table 4.1. There were no significant differences in cumulative precipitation in each period. The range of period cumulative precipitations was 175–313 mm in the G1 period, 257–522 mm in the G2 period, and 112–565 mm in the G3 period. Mean daily precipitation in the G1, G2, and G3 periods ranged from 1.9 to 8.3 mm·d⁻¹, and there was no significant difference among the periods. The annual precipitation ranged from 883 to 1526 mm.
Fig. 4.1 Seasonal variation in meteorological variables in the fertilizer (F) and manure (M) plots; precipitation (a), daily air temperature (Ta) (b), incident of photosynthetically active radiation (PAR) (c).

The daily mean air temperature increased from February and reached the maximum temperature in August before decreasing gradually thereafter (Fig. 4.1). The patterns of daily air temperature were similar across all years. There were significant differences in mean air temperature among the periods ($p < 0.01$) (Table 4.1). Although there was no significant difference in mean air temperature between the G2 and G3 periods (17.1–19.9°C in G2 and 13.8–18.9°C in G3, respectively), air temperatures in the G2 and G3 periods were significantly higher than that in the G1 period (8.4–0.6°C). The annual mean air temperature ranged from 7.2 to 7.7 °C.
Table 4.1 Mean air temperature (Ta), cumulative precipitation (P) and cumulative photosynthetically active radiation (PAR). NG is non-growing season. G1 is the period from the beginning of the growing season to the first crop harvest, G2 is the period from the first harvest to the second harvest and G3 is the period from the second harvest to third harvest, and G4 is the period from the third harvest to the end of the growing season.

<table>
<thead>
<tr>
<th>Year</th>
<th>Period</th>
<th>Start date</th>
<th>End date</th>
<th>Days</th>
<th>Ta (°C)</th>
<th>P (mm)</th>
<th>PAR (mol·m⁻²)</th>
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</thead>
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<td>Nov. 2, 2011</td>
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<td>Nov. 3, 2012</td>
<td>367</td>
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Result of one way repeated measures ANOVA during growing season (G1, G2 and G3 periods)

<table>
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<th>d.f.</th>
<th>Ta</th>
<th>F</th>
<th>p</th>
<th>P</th>
<th>F</th>
<th>p</th>
<th>PAR</th>
<th>F</th>
<th>p</th>
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The daily PAR increased from January to May, and then decreased gradually (Fig. 4.1). Similar patterns of daily PAR were recorded during the 5-year study period. The cumulative PAR in each growing season period ranged from 1411 to 2198 mol·m⁻² in the G1 period, from 1740 to 2337 mol·m⁻² in the G2 period, and from 1477 to 2043 mol·m⁻² in the G3 period. There was no significant difference in the cumulative PAR between the periods (Table 4.1). However, there was significant difference in mean PAR ($p < 0.01$) among periods, and the seasonal mean PAR in the G1 season was highest (31.9–34.3 mol·m⁻²·d⁻¹), followed by that in the G2 period (26.4–32.0 mol·m⁻²·d⁻¹) and G3 period (20.0–27.0 mol·m⁻²·d⁻¹). The annual cumulative PAR ranged from 7570 to 8557 mol·m⁻²·yr⁻¹.

### 4.2.2 Seasonal change of NEP, GPP and RE

The daily NEP showed a similar seasonal pattern throughout the year (Fig. 4.2). Daily NEP increased rapidly from April when grass started growing, and reached the maximum NEP just before the first crop harvest. The maximum daily NEP during the G1 period ranged from 7.7 to 12.8 g C m⁻²·d⁻¹ in the F plot, and from 7.8 to 11.7 g C m⁻²·d⁻¹ in the M plot. The daily NEP just after the first crop harvest was negative, which indicates that CO₂ was emitted from the ecosystem. However, CO₂ uptake began about 2 weeks after the first crop harvest, and the maximum daily NEP during the G2 period ranged from 8.4 to 10.7 g C m⁻²·d⁻¹ in the F plot, and from 6.7 to 8.5 g C m⁻²·d⁻¹ in the M plot. The seasonal change of daily NEP after the second crop harvest was similar to that after the first crop harvest, but the maximum daily NEP during the G3 period was lower than during the G1 and G2 periods. The daily GPP and RE also increased from April (Fig. 4.2). The daily GPP dropped sharply after harvest, while daily RE did not change significantly after harvest.
Fig. 4.2 Seasonal variation in net ecosystem production (NEP) (a), gross primary production (GPP) (b) and ecosystem respiration (RE) (c). The up arrows indicate the timing of harvest and the down arrows indicate the timing of manure application.
Table 4.2 Seasonal net ecosystem production (NEP), gross primary production (GPP), ecosystem respiration (RE) and harvest in the fertilizer (F) and manure (M) plots (Mg C ha$^{-1}$ per period). NG is non-growing season. G1 is the period from the beginning of the growing season to the first crop harvest, G2 is the period from the first harvest to the second harvest, G3 is the period from the second harvest to third harvest, and G4 is the period from the third harvest to the end of the growing season. The start and end date for each season was shown in Table 4.1.

<table>
<thead>
<tr>
<th>Year</th>
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<th>GPP</th>
<th>RE</th>
<th>Harvest</th>
</tr>
</thead>
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<td></td>
<td></td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
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<tr>
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<td>3.9</td>
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Continued
Table 4.2 continue

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<th>RE</th>
<th>Harvest</th>
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Result of two way repeated measures ANOVA during growing season (G1, G2 and G3 periods)

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<th>Harvest</th>
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<td>0.0</td>
</tr>
<tr>
<td>Season</td>
<td>2</td>
<td>10.1</td>
<td>&lt;0.01</td>
<td>9.8</td>
</tr>
<tr>
<td>Treatment × Season</td>
<td>2</td>
<td>11.0</td>
<td>&lt;0.01</td>
<td>4.5</td>
</tr>
</tbody>
</table>
The cumulative NEP, GPP, and RE across the study period are shown in Table 4.3. During the growing season, there was a significant difference in the cumulative NEP among the treatment plots and periods ($p < 0.01$), and there was a significant treatment-by-season interaction ($p < 0.01$). The NEP in the G1 period (1.9 ± 0.3 Mg C ha$^{-1}$ per period in the F plot, and 1.8 ± 0.4 Mg C ha$^{-1}$ per period in the M plot) was the highest. This was followed by G2 period (1.3 ± 0.5 Mg C ha$^{-1}$ per period in the F plot, and 0.8 ± 0.6 Mg C ha$^{-1}$ per period in the M plot) and G3 period (0.7 ± 0.3 Mg C ha$^{-1}$ per period in the F plot, and 0.5 ± 0.4 Mg C ha$^{-1}$ per period in the M plot). The cumulative NEP was significantly greater in F plots than in M plots in the G2 period, and did not differ significantly between the F and M plots in the G1 and G3 periods. Annual NEP was 3.4 ± 0.4 and 2.4 ± 0.6 Mg C ha$^{-1}$·yr$^{-1}$ in the F and M plots, respectively, and there was a significant difference between the treatment plots ($p < 0.01$). The result of multiple regression analysis showed that the mean NEP was significantly correlated with mean air temperature, daily precipitation, and daily PAR throughout the study period, following the equation: \( \text{NEP} = -0.080 - 0.087 \times \text{air temperature} + 0.098 \times \text{precipitation} + 0.068 \times \text{PAR} \) ($R^2 = 0.69$, $p < 0.01$). The annual NEP was 3.2 ± 0.5 and 2.2 ± 0.5 Mg C ha$^{-1}$·yr$^{-1}$ in the F and M plots, respectively, and there was a significant difference between the treatment plots ($p < 0.01$) (Table 4.3).

There was a significant difference in the cumulative GPP among the periods ($p < 0.01$), as well as significant treatment-by-period interactions ($p < 0.05$). However, there was no significant difference among the treatments. The cumulative GPP was significantly greater in the G2 period (6.7 ± 0.7 Mg C ha$^{-1}$ per period in the F plot, and 6.4 ± 0.8 Mg C ha$^{-1}$ per period in the M plot) than in the G1 (4.0 ± 0.2 Mg C ha$^{-1}$
per period in the F plot, and 4.2 ± 0.3 Mg C ha\(^{-1}\) per period in the M plot) and G3 (5.1 ± 1.1 Mg C ha\(^{-1}\) per period in the F plot, and 5.3 ± 1.3 Mg C ha\(^{-1}\) per period in the M plot) periods in both plots. Multiple regression analysis showed that the mean GPP was correlated with the mean air temperature and daily precipitation across the periods, following the equation; GPP = 1.61 + 0.21 \times \text{air temperature} + 0.14 \times \text{precipitation} (R^2 = 0.46, p < 0.01). There was no significant difference in annual GPP (17.1 ± 0.7 and 17.2 ± 1.1 Mg C ha\(^{-1}\)·yr\(^{-1}\) in the F and M plots, respectively) between the treatments (Table 4.3).

There was a significant difference in the cumulative RE among the treatment plots and periods (p < 0.01), and there was no significant treatment-by-period interaction. The cumulative RE was significantly greater in the M plot than in the F plot. The RE in the G1 period (2.2 ± 0.2 Mg C ha\(^{-1}\) per period in the F plot, and 2.4 ± 0.3 Mg C ha\(^{-1}\) per period in the M plot) was significantly lower than those in the G2 (5.3 ± 0.8 Mg C ha\(^{-1}\) per period in the F plot, and 5.7 ± 1.1 Mg C ha\(^{-1}\) per period in the M plot) and G3 (4.4 ± 1.0 Mg C ha\(^{-1}\) per period in the F plot and 4.8 ± 1.1 Mg C ha\(^{-1}\) per period in the M plot) periods. There was no significant difference between the G2 and G3 periods. Multiple regression analysis showed that the mean RE was significantly correlated with the mean air temperature and daily PAR throughout the study period, by using the following equation: RE = 1.83 + 0.30 \times \text{air temperature} – 0.0709 \times \text{PAR} (R^2 = 0.72, p < 0.01). The mean annual RE in the M plot (14.8±1.0 Mg C ha\(^{-1}\)·yr\(^{-1}\)) was significantly greater than that in the F plot (13.7±0.7 Mg C ha\(^{-1}\)·yr\(^{-1}\)) (Table 4.3).
4.2.3 Heterotrophic respiration

The seasonal change of soil surface CO\textsubscript{2} flux of RHs from the root exclusion plot is presented in Fig. 4.3. The RHs increased rapidly from April with the increase of temperature, and reached the maximum CO\textsubscript{2} flux in July, then decreased with the decrease of temperature. There was a significant exponential correlation between the RHs and soil temperature at 5 cm depth (Fig. 4.4).

Fig. 4.3 Seasonal variation in soil surface CO\textsubscript{2} flux in the root exclusion plots (RHs) established in fertilizer plot (F) from 2011 to 2012. Data are represented as mean ± SD.

Fig. 4.4 Relationship between soil surface CO\textsubscript{2} flux (RHs) and soil temperature in the fertilizer (F) plot from 2011 to 2012. The line indicates an exponential regression. Data are represented as mean ± SD.

\[ y = 53.837e^{0.86(156.02-11273.15-227.13)} \]
\[ (R^2 = 0.62, \ p < 0.01) \]
There was no significant difference in the cumulative RHs among the periods (Table 4.4). However, the RHs in the G1 period tended to be significantly smaller than those in the G2 and G3 periods. The annual RHs in the F and M plots ranged from 4.5 to 5.1 Mg C ha⁻¹·yr⁻¹. RHm in managed grassland was estimated as the difference between the value of RE in M and F plot (Eq. 3.6). Annual RHm was 1.3 and 1.2 Mg C ha⁻¹·yr⁻¹ in 2011 and 2012, respectively (Table 4.4).

The relationships between RH (RH = RHs + RHm) and RE in both F and M plots are shown in Fig. 4.5. There was a significant positive correlation between the RH and RE in both plots (p < 0.01). The contribution of the annual RH to RE in 2011 and 2012 was 31% and 33% in the F plot, and 40% and 42% in the M plot, respectively.

![Fig. 4.5 The relationship between ecosystem respiration (RE) and heterotrophic respiration in fertilizer plot (RHs) (a) and manure plot (RH) (b) from 2011 to 2012.](image)
Table 4.3  Annual net ecosystem production (NEP), gross primary production (GPP), ecosystem respiration (RE), ANPP, BNPP, heterotrophic respiration (RH), harvest, net biome production (NBP) and heterotrophic respiration of manure (RHm) in fertilizer plot (F), and manure plot (M) in the managed grassland (Mg C ha\(^{-1}\)·yr\(^{-1}\)) and the statistical result of one way ANOVA with repeated measures.

<table>
<thead>
<tr>
<th>Year</th>
<th>NEP F</th>
<th>NEP M</th>
<th>GPP F</th>
<th>GPP M</th>
<th>RE F</th>
<th>RE M</th>
<th>Harvest F</th>
<th>Harvest M</th>
<th>NBP F</th>
<th>NBP M</th>
<th>RHm M</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008</td>
<td>3.2</td>
<td>2.6</td>
<td>16.7</td>
<td>16.9</td>
<td>13.4</td>
<td>14.3</td>
<td>4.3</td>
<td>4.3</td>
<td>-1.1</td>
<td>0.4</td>
<td>0.9</td>
</tr>
<tr>
<td>2009</td>
<td>3.9</td>
<td>2.7</td>
<td>16.7</td>
<td>15.9</td>
<td>12.8</td>
<td>13.3</td>
<td>3.2</td>
<td>3.2</td>
<td>0.7</td>
<td>1.8</td>
<td>0.5</td>
</tr>
<tr>
<td>2010</td>
<td>2.8</td>
<td>1.7</td>
<td>16.6</td>
<td>17.1</td>
<td>13.8</td>
<td>15.3</td>
<td>2.6</td>
<td>2.3</td>
<td>-0.2</td>
<td>1.3</td>
<td>1.5</td>
</tr>
<tr>
<td>2011</td>
<td>3.7</td>
<td>3.1</td>
<td>18.2</td>
<td>19.0</td>
<td>14.6</td>
<td>15.9</td>
<td>4.2</td>
<td>4.7</td>
<td>-0.5</td>
<td>0.4</td>
<td>1.3</td>
</tr>
<tr>
<td>2012</td>
<td>3.3</td>
<td>1.8</td>
<td>17.1</td>
<td>17.0</td>
<td>13.8</td>
<td>15.0</td>
<td>3.1</td>
<td>2.8</td>
<td>0.2</td>
<td>0.3</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Average ± SD 3.4 ± 0.4 2.4 ± 0.6 17.1 ± 0.7 17.2 ± 1.1 13.7 ± 0.7 14.8 ± 1.0 3.3 ± 0.7 3.5 ± 1.0 -0.1 ± 0.8 0.8 ± 0.7 1.1 ± 0.4

Result of one way repeated measures ANOVA

<table>
<thead>
<tr>
<th>d.f.</th>
<th>NEP F p</th>
<th>GPP F p</th>
<th>RE F p</th>
<th>Harvest F p</th>
<th>NBP F p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1   32.3 &lt;0.01</td>
<td>0.2  0.685</td>
<td>38.4 &lt;0.01</td>
<td>0.2  0.90</td>
<td>16.5 &lt;0.05</td>
</tr>
</tbody>
</table>
Table 4.4 Heterotrophic respiration of soil (RHs) and manure (RHm), aboveground net primary production (ANPP), belowground net primary production (BNPP) estimated BNPP (BNPP\textsubscript{est}) and estimated NPP (NPP\textsubscript{est}) in the fertilizer (F) and manure (M) plots (Mg C ha\textsuperscript{-1} per period). NG is non-growing season. G1 is the period from the beginning of the growing season to the first crop harvest, G2 is the period from the first harvest to the second harvest and G3 is the period from the second harvest to third harvest. The start and end date for each season was shown in Table 4.1.

<table>
<thead>
<tr>
<th>Year</th>
<th>Period</th>
<th>RHs F</th>
<th>RHs M</th>
<th>ANPP F</th>
<th>ANPP M</th>
<th>BNPP\textsubscript{bio} F</th>
<th>BNPP\textsubscript{bio} M</th>
<th>BNPP\textsubscript{est} F</th>
<th>BNPP\textsubscript{est} M</th>
<th>NPP\textsubscript{est} F</th>
<th>NPP\textsubscript{est} M</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011</td>
<td>NG</td>
<td>0.5</td>
<td>0.7</td>
<td>0.0</td>
<td>-0.2 (0.0)</td>
<td>-0.2 (0.2)</td>
<td>-0.2 (0.8)</td>
<td>-</td>
<td>-</td>
<td>0.1</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>G1</td>
<td>0.5</td>
<td>0.6</td>
<td>0.2</td>
<td>2.4 (0.1)</td>
<td>2.6 (0.5)</td>
<td>2.0 (2.4)</td>
<td>-</td>
<td>-</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>G2</td>
<td>1.5</td>
<td>1.5</td>
<td>0.6</td>
<td>1.1 (0.1)</td>
<td>1.1 (0.4)</td>
<td>-1.1 (2.4)</td>
<td>-</td>
<td>-</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>G3</td>
<td>2.0</td>
<td>2.1</td>
<td>0.5</td>
<td>1.0 (0.1)</td>
<td>1.1 (0.3)</td>
<td>0.3 (2.5)</td>
<td>-</td>
<td>-</td>
<td>1.9</td>
<td>2.8</td>
</tr>
<tr>
<td>2012</td>
<td>NG</td>
<td>0.6</td>
<td>0.8</td>
<td>0.2</td>
<td>0.0 (0.0)</td>
<td>0.1 (0.2)</td>
<td>-1.8 (2.5)</td>
<td>-2.1 (0.6)</td>
<td>0.0</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G1</td>
<td>0.6</td>
<td>0.7</td>
<td>0.2</td>
<td>1.9 (0.1)</td>
<td>1.5 (0.2)</td>
<td>0.0 (0.6)</td>
<td>0.0 (0.2)</td>
<td>0.7</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G2</td>
<td>1.7</td>
<td>2.0</td>
<td>0.2</td>
<td>1.4 (0.0)</td>
<td>1.3 (0.2)</td>
<td>-0.1 (0.5)</td>
<td>0.2 (0.2)</td>
<td>1.9</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G3</td>
<td>1.6</td>
<td>1.6</td>
<td>0.6</td>
<td>0.8 (0.0)</td>
<td>0.7 (0.2)</td>
<td>-</td>
<td>-</td>
<td>1.2</td>
<td>1.4</td>
<td></td>
</tr>
</tbody>
</table>

2011 Annual 4.5 4.9 1.3 4.3 (0.1) 4.5 (0.8) 1.1 (4.3) - 3.9 5.0 8.2 9.5
2012 Annual 4.5 5.1 1.2 4.0 (0.1) 3.5 (0.4) -1.8 (2.6)* -1.8 (0.6)* 3.7 4.7 7.7 8.2

ANPP and BNPP represent means ± (uncertainties/100 × means).
* Annual BNPP in 2012 showed the sum of BNPP in NG, G1 and G2 periods.
4.2.4 Net primary production

Aboveground biomass significantly increased from the beginning to the end of each period (Fig. 4.6). The belowground biomass ranged from 0.5 to 3.5 Mg C ha$^{-1}$ in the F plot and from 0.3 to 2.7 Mg C ha$^{-1}$ in the M plot (Fig. 4.6). Belowground biomass was not significantly different between periods.

There was a significant difference in the harvest among periods ($p < 0.05$), but there was no significant difference between the treatments, or treatments-by-period interactions. Harvest in the G3 period (0.7–1.3 and 0.7–1.1 Mg C ha$^{-1}$ per period in the F and M plots, respectively) tended to be smaller than those in the G1 and G2 periods (Table 4.2). The annual harvest was 3.3 ± 0.6 and 3.2 ± 0.8 Mg C ha$^{-1}$·yr$^{-1}$ in the F and M plots, respectively, and there was no significant difference in the annual harvest between treatments (Table 4.3).

![Fig. 4.6 Seasonal change of aboveground and belowground biomass in fertilizer plot (F) and manure plot (M). Data are represented as mean ± SD.](image-url)
The ANPP, BNPP\textsubscript{bio} and BNPP\textsubscript{est} in 2011 and 2012 are shown in Table 4.4. Annual ANPP for the F and M plots ranged from 4.0 to 4.5 Mg C ha\textsuperscript{-1}\cdot yr\textsuperscript{-1}, and annual BNPP\textsubscript{bio} ranged from -1.8 to 1.1 Mg C ha\textsuperscript{-1}\cdot yr\textsuperscript{-1}. Uncertainties ranged from 2% to 18% for annual ANPP, and from 33% to 391% for annual BNPP\textsubscript{bio}. There was no significant difference in BNPP\textsubscript{est} between the treatments and periods. However, BNPP\textsubscript{est} in the G2 period tended to be greater than BNPP\textsubscript{est} in the G1 period. The values of annual BNPP\textsubscript{est} in 2011 and 2012 were 3.9 and 3.7 Mg C ha\textsuperscript{-1}\cdot yr\textsuperscript{-1} for the F plot, and 5.0 and 4.7 Mg C ha\textsuperscript{-1}\cdot yr\textsuperscript{-1} for the M plot, respectively, and these were significantly larger than annual BNPP\textsubscript{bio} ($p < 0.05$).

The NPP\textsubscript{est} in G1, G2 and G3 period ranged from 2.0 to 3.3 Mg C ha\textsuperscript{-1} per period in the F plot, and from 2.1 to 3.8 Mg C ha\textsuperscript{-1} per period in the M plot. There was no significant difference in the contribution of BNPP\textsubscript{est} to NPP\textsubscript{est} (BNPP\textsubscript{est}/NPP\textsubscript{est}) among periods and treatment plots. However, BNPP\textsubscript{est}/NPP\textsubscript{est} in the G2 (0.56–0.58 and 0.56–0.61 in the F and M plots, respectively) and G3 period (0.60–0.66 and 0.67–0.74 in the F and M plots, respectively) tended to be higher than that in the G1 period (0.14–0.27 and 0.13–0.46 in the F and M plots, respectively). The annual BNPP\textsubscript{est}/NPP\textsubscript{est} in 2011 and 2012 was 0.47 and 0.48 for the F plot, and 0.52 and 0.57 for the M plot, respectively.

4.2.5 NBP

Annual NBP is shown in Table 4.5. The annual NBP was -0.1 ± 0.7 and 0.8 ± 0.7 Mg C ha\textsuperscript{-1}\cdot yr\textsuperscript{-1} in the F and M plots, respectively. There was a significant difference between the treatment plots ($p < 0.05$).
4.3 Discussion

4.3.1 Effect of management practice on C dynamics and budgets

There was no significant difference in annual GPP and harvest between the F and M plots (Table 4.2). This indicates that application of manure alongside chemical fertilizer did not reduce grass production compared with application of chemical fertilizer alone. However, the RE was significantly higher in the M plot than in the F plot (Table 4.2). This would be due to RH of manure (RHm) in the M plot, and the RHm can be estimated as the difference of RE between the M and F plots. The annual RHm from 2010 to 2012 was larger than that in 2008 and 2009 (Table 4.4). This indicates that manure applied in the grassland was still being decomposed more than two years after application. Bhogal et al. (2011) have reported that improvements in soil quality and functioning after repeated additions of livestock manures can persist for more than two years after the cessation of applications in England.

The annual RHm of 1.3 ± 0.1 Mg C ha\(^{-1}\)·yr\(^{-1}\) accounted for 40% of manure C applied (Table 4.4). Lee et al. (2007) estimated that approximately 45% of added C with cattle manures application for four years in Switch grass grassland of South Dakota was respired. Matsuura et al. (2014) reported similar values of RHm ranging from 0.5 to 1.3 Mg C ha\(^{-1}\)·yr\(^{-1}\) with an average of 0.8 Mg C ha\(^{-1}\)·yr\(^{-1}\) from grassland following application of cattle manure for three-years in Tochigi, Japan. The total amount of C in applied manure reported by Matsuura et al. (2014) ranged from 2061 to 4207 kg C ha\(^{-1}\)·yr\(^{-1}\), and the rate of decomposition was about 23% during the three years. The different decomposition rate reported by Matsuura et al. (2014) compared with the present study might be due to differences in the C/N ratio of manure. The C/N ratio of manure used by Matsuura et al. (2014) was 24.4 ± 4.8, while that used by us was 13.5 ± 4.3. It is well known that the decomposition rate of organic matter with
A high C/N ratio is generally low (Eiland et al., 2001; Toma and Hatano; 2007; Vigil and Kissel, 1991).

NBP in the M plot was significantly larger than that in the F plot (Table 4.3). The managed grassland in the M plot acted as C sink, which was confirmed by the positive value of NBP during the 5-years of this study, while the NBP in the F plot was negative, indicating that the ecosystem was acting as a carbon source. A similar result was obtained by Ammann et al. (2007), who reported that the NBP (NBP = – NEE – harvest biomass export + carbon input through organic fertilization) was 1.5 and -0.6 Mg C ha\(^{-1}\)·yr\(^{-1}\) in intensively managed (manure and fertilizer application) and extensively managed (no application of manure and fertilizer) agricultural grassland in Switzerland respectively. These results indicate that manure application can enhance the annual NBP.

### 4.3.2 Estimates of BNPP

In this study, RHs was estimated using root exclusion technique, and RH was calculated as the sum of RHs and RHm. Byrne and Kiely (2006) measured RE in eight plots with grass cover and RH in five plots with grass removed using chamber method in an intensively managed grassland in Ireland with slurry was applied, and reported RH/RE ratio of 0.56. This is higher than the ratio of this study. However, Ciais et al. (2010) reported RH/RE ratio of 0.40 in European grasslands, in which RE was measured using eddy covariance measurement, and RH was estimated from mass balance calculation. Kosugi et al. (2010) found RH/RE ratio of 0.28 at 25 °C using the chamber method in a managed C\(_3\) turfgrass field in Japan. These values are comparable to the values obtained in this study.
In this study, RH of leaf litter and root litter were not measured. However, the decreased amount of litter from the aboveground and belowground parts was included in ANPP and BNPP\textsubscript{bio} in this study, because the biomass was measured together with the dead parts of the previous period. The annual BNPP\textsubscript{bio} in 2012 showed negative values, even though the uncertainties were large (Table 4.4). Shimizu \textit{et al.} (2009) also reported that the belowground biomass ranged from 8 to 11 Mg C ha\textsuperscript{-1} throughout the year without distinct seasonal change in a managed grassland of reed canary grass in Hokkaido, and the annual BNPP was -1.2±8.4 Mg C ha\textsuperscript{-1} yr\textsuperscript{-1}. This negative value would indicate that decreased amount of root litter is faster than the production amount of root. In this study, uncertainties in BNPP\textsubscript{est} were not able to be directly calculated. However, Dragoni \textit{et al.} (2007) reported that random uncertainties in annual NEP estimated using eddy covariance flux measurement over a mixed deciduous forest varied from 3-4%. Even though there is potential for the effect of systematic bias in the measured eddy covariance flux, annual BNPP\textsubscript{est} showed positive values (Table 4.4), and was significantly larger than annual BNPP\textsubscript{bio} ($p < 0.05$). This indicates that the root litter might still remain in soil, even though some of them were decomposed as CO\textsubscript{2}. Carbon leaching loss was not estimated in this study. However, Kindler \textit{et al.} (2011) reported that leaching of biogenic dissolved inorganic C was 0.24±0.07 Mg C ha\textsuperscript{-1} yr\textsuperscript{-1}, and dissolved organic C leaching was 0.05±0.02 Mg C ha\textsuperscript{-1} yr\textsuperscript{-1} for grasslands, which are extremely lower than BNPP\textsubscript{est} in this study. \[\text{BNPP}_{\text{est}}/\text{NPP}_{\text{est}}\] in the G2 and G3 period tended to be higher than that in the G1 period in this study. This indicates that the allocation rate of photosynthetic products to aboveground biomass was larger in the G1 period than in other periods. The peak of aboveground biomass was found in G1 period when the flush of rapid growth was happening in spring. This is thought to be because the remobilization from the
belowground biomass (Xiong et al., 2009). The higher $\text{BNPP}_{\text{est}}/\text{NPP}_{\text{est}}$ in the G2 and G3 periods indicates that the allocation of photosynthetic products to belowground biomass was larger. This is attributed to the enhanced proportion of photosynthetic products allocated to belowground biomass for overwintering. In an early review on belowground structures of grasses, Weinmann (1948) suggested that the roots or rhizomes of grasses act as a storage organ during winter. Xiong et al. (2009) reported that reed canary grass in northern Sweden exhibited low rhizome growth in June when the re-shooting had just started, after which growth increased steadily during the growing season and reached a peak sometime in late autumn and remained high until the next spring. Miscanthus (Himken et al., 1997) and common reed (Granéli et al., 1992) showed similar seasonal dynamics in their roots and rhizomes. These seasonal patterns may be attributed to the mobilization of rhizome carbohydrates and mineral nutrient stores to support re-growth in the spring and translocation of carbohydrates and mineral nutrients at the end of the growing season.

The annual $\text{BNPP}_{\text{est}}/\text{NPP}_{\text{est}}$ ranged from 0.47 to 0.57 in this study. Cahill et al. (2009) estimated BNPP by using five methods, in which three of them are based on models predicting root-turnover proposed by Gill et al. (2002), which are direct function of ANPP method (DIR), constant function method (CON), exponential function of mean annual temperature method (MAT), and two of them are based on direct observations of root turnover, which are minirhizotron method (MRH) (Brye et al., 2002) and root window method (RW) (Hayes and Seastedt, 1987) in prairie restoration ($Bromus inermis$ Leyss) in southwestern Wisconsin. The ratio of BNPP/NPP estimated by these models were 0.27 for DIR, 0.2 for CON and 0.14 for MAT, and those from direct observations were 0.59 for MRH and 0.17 for RW. Among the values of BNPP/NPP ratio from different methods, the value of 0.59 from
MRH method was closest to the value in this study, which was obtained indirectly based on the ecosystem carbon budget. Cahill et al. (2009) suggested that turnover estimate by MRH method might be the outlier, but could represent the best estimate of prairie root dynamics, because MRH method used the video image with high resolution taken by high frequency which can detect rapid growth and death of fine roots.

4.4 Conclusion

The C budget was estimated for five years in two experimental plots established in a managed grassland: one receiving only chemical fertilizer and the other receiving composted cattle manure and supplementary chemical fertilizer. There was a significant difference in seasonal NEP and RE between the treatment plots, while there was no significant difference in GPP and harvest. This indicates that combined application of manure and chemical fertilizer did not reduce grass production compared with application of chemical fertilizer only, but manure application would enhance RE through manure decomposition. However, long-term manure application can enhance the annual NBP. In both treatments, root litters may remain in the soils, and become significant contributor to soil C stock in the grassland.
Chapter 5. Land use change effect on carbon budget: from managed grassland to corn field

5.1 Introduction

Current and past land use practices are critical in determining the distribution and size of global terrestrial C sources and sinks (Canadel JG., 2002). Land use type is an important factor to control C storage, and shifts from one type to another are responsible for large C fluxes in and out of the terrestrial ecosystem. Land use change (LUC) in agriculture is generally associated with changes in vegetation in an ecosystem, and thus affects the cycling and storage of C (Guo and Gifford, 2002), which results in C emissions to the atmosphere (IPCC, 2000; McGuire et al., 2001; Houghton et al., 2012). The total emission of C associated with deforestation and fossil-fuel burning in the period 1985-1990 was about 450 PgC, of which 180-200 PgC was from the land use change, and CO₂ emission contribute about 10-30% of the total anthropogenic C (Houghton et al., 1999, Defries et al., 1999).

Most changes in land uses affect the vegetation and soil of an ecosystem and thus change the amount of C. The changes may be large, for example, with the conversion of forest to cropland. A meta-analysis of 74 studies revealed that conversion from grassland to cropland resulted in the largest loss of soil C (59%) followed by the conversion of native forest to crops (42%) (Guo and Gifford, 2002). Land use changes inevitably influence the structure and species composition of plant communities that alter the nature of litter, induce variation in the soil physical properties and cause photosynthesis changes, all of which ultimately affect root respiration (RR) and soil respiration (RS) (Hopkins et al., 2013; Emran et al., 2012).
Management practices and climatic conditions could affect the ecosystem’s ability to being either a source or sink of C (Peng et al., 2009; Sheng et al., 2009; Shimizu et al., 2009). Some management practices could increase the organic C in the soil (e.g., manure application) while others could remove or decrease organic C (e.g. tillage). The knowledge about effect of land use change with continuous manure application treatment on the C budget is still limited. In this study, after five consecutive years of managed grassland, the research site was converted a to corn field with continuous manure application.

The objectives of this study was to find out the effect of land use conversion from managed grassland to corn field on the various components [C input (manure application) and output (RH, RS, NPP, harvest] of the C budget.

5.2 Results

5.2.1 Environmental variables

The daily precipitation is shown in Fig. 5.1, and the cumulative precipitation for each year is shown in Table 5.1. The cumulative annual precipitation ranged from 883 to 1526 mm during the five years in managed grassland and from 1240 to 1355 mm during two years in corn field. The daily mean air temperature increased from February and reached a maximum temperature in August before decreasing gradually thereafter (Fig. 5.1). The patterns of daily air temperature were similar across all years. The daily PAR increased from January to May, and then decreased gradually (Fig. 5.1). Similar patterns of daily PAR were recorded during the 7-year study period. The annual cumulative PAR ranged from 7570 to 8720 mol·m⁻²·yr⁻¹.
Fig. 5.1 Seasonal variation in precipitation (a), daily air temperature (Ta) (b), daily soil temperature at 5 cm depth (c) and photosynthetically active radiation (PAR) (d) in managed grassland (2008 to 2012) and corn field (2013 to 2014).
Table 5.1 Annual mean air temperature (Ta), cumulative precipitation (P), cumulative photosynthetically active radiation (PAR) and mean soil temperature at 5 cm depth (Ts).

<table>
<thead>
<tr>
<th>Year</th>
<th>Star date</th>
<th>End date</th>
<th>Days</th>
<th>Ta (°C)</th>
<th>P (mm)</th>
<th>PAR (mol·m⁻²·yr⁻¹)</th>
<th>Ts (°C)</th>
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</thead>
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<tr>
<td>2008</td>
<td>Nov. 17, 2007</td>
<td>Nov. 18, 2008</td>
<td>368</td>
<td>7.2</td>
<td>883</td>
<td>8557</td>
<td>8.3</td>
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<tr>
<td>2009</td>
<td>Nov. 19, 2008</td>
<td>Nov. 14, 2009</td>
<td>361</td>
<td>7.7</td>
<td>1367</td>
<td>8226</td>
<td>9.1</td>
</tr>
<tr>
<td>2010</td>
<td>Nov. 15, 2009</td>
<td>Nov. 15, 2010</td>
<td>366</td>
<td>7.7</td>
<td>1526</td>
<td>7570</td>
<td>9.4</td>
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<tr>
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<td>Nov. 2, 2011</td>
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<td>1389</td>
<td>8183</td>
<td>9.6</td>
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<tr>
<td>2012</td>
<td>Nov. 3, 2011</td>
<td>Nov. 11, 2012</td>
<td>367</td>
<td>7.3</td>
<td>1180</td>
<td>8473</td>
<td>9.9</td>
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<tr>
<td>2013</td>
<td>Nov. 4, 2012</td>
<td>Nov. 4, 2013</td>
<td>366</td>
<td>6.9</td>
<td>1355</td>
<td>7730</td>
<td>9.6</td>
</tr>
</tbody>
</table>

5.2.2 NEP, GPP and RE

The general pattern of NEP in both managed grassland and corn field followed a similar seasonal cycle with most photosynthesis occurring in spring and summer when incoming solar radiation was greatest (Fig. 5.2). In the managed grassland, the daily NEP had a similar pattern throughout the year with the highest rate of net C uptake occurring from the end of May to September in all the five years from 2008 to 2012 (7.6 to 11.7 g C m⁻²·d⁻¹). The daily NEP in corn field also showed a similar pattern in both years of the study, with the highest C uptake occurring in the middle of July (16.2 to 21.1 g C m⁻²·d⁻¹). The daily NEP quickly decreased after harvest following the reduction of GPP, while RE was not affected at all (Fig. 5.4). Herbicide application, similar to harvest, also led to decreased GPP and NEP. However the reduction of NEP and GPP after herbicide application was slower, taking a few days to reach the same level as that following harvesting (Fig. 5.3). The annual NEP in managed grassland ranged from 1.6 to 2.7. In corn field, the annual NEP in 2014 was greater than that in 2013. The annual NEP in corn field was 1.2 and 4.3 Mg C ha⁻¹·yr⁻¹ in 2013 and 2014, respectively. Herbicide application during the conversion from managed grassland to corn field in 2012 led to a decrease of the NEP as the C source (Fig. 5.3).
The peak of daily GPP in corn field occurred in July and August from 2013 to 2014 (27.9 to 32.9 g C m$^{-2}$·d$^{-1}$) and it was higher than in managed grassland which ranged from 15.3 to 19.0 g C m$^{-2}$·d$^{-1}$ and occurred in June and July from 2008 to 2012. Although peak of daily GPP was greater in corn field than in managed grassland due to canopy architecture and C4-type photosynthesis, the annual average
GPP was lower in the corn field than in the managed grassland due to shorter growing period in the corn field (160 days in corn field and 198 days in managed grassland). The annual average GPP was $17.2 \pm 1.1$ and $16.1 \pm 1.8 \text{ Mg C ha}^{-1} \cdot \text{yr}^{-1}$ in managed grassland and corn field, respectively. The increase of RE occurred when air temperature was higher than 5°C. The daily RE had a similar pattern in both managed grassland to corn field. The daily RE in 2013 and 2014 rapidly increased after tillage practices following the reduction of NEP (Fig. 5.2) The annual average RE in managed grassland ($14.9 \pm 1.1 \text{ Mg C ha}^{-1} \cdot \text{yr}^{-1}$) was slightly higher than in corn field ($13.3 \pm 0.5 \text{ Mg C ha}^{-1} \cdot \text{yr}^{-1}$).

![Fig. 5.3 Daily variation in net ecosystem production (NEP) (black color line), gross primary production (GPP) (blue color line) and ecosystem respiration (RE) (green color line) from October 2012 to November 2012. The dashed red line indicated the timing of herbicide application during the conversion from managed grassland to corn field.](image-url)
5.2.3 Heterotrophic and soil respiration

The seasonal change of soil surface CO₂ flux from the root exclusion plot (Heterotrophic respiration, RH) is presented in Fig. 5.5. The RH in managed grassland and corn field had a similar pattern, whereby it increased rapidly from April with the increase of temperature, and reached the maximum CO₂ flux in July, then decreased with the decrease of temperature. There was a significant positive correlation between RH and soil temperature at 5 cm depth (Fig. 5.6). There was no difference of Q₁₀ value of RHs in managed grassland and corn field (Table 5.2). The Q₁₀ of RH in the corn field was 1.6, while there was no Q₁₀ value of RH in managed grassland. The RE increased with the increase in RH. The annual average RH was 6.4 ± 0.0 and 8.1 ± 0.0 Mg C ha⁻¹·yr⁻¹ in managed grassland and corn field respectively (Table 5.2). The contribution of the annual RH to RE in corn field (61%) was greater
than in managed grassland (42%) (Table 5.3). The RE decreased with the decrease in RA. The annual average RA was $9.1 \pm 0.6$ and $5.2 \pm 0.4 \text{ Mg C ha}^{-1}\cdot\text{yr}^{-1}$ in managed grassland and corn field, respectively. The contribution of the annual RA to RE in managed grassland (58%) was greater than in corn field (39%) (Table 5.2).

Fig. 5.5 Seasonal variation of soil surface CO$_2$ flux (RHs and RH) in the root exclusion plots established in managed grassland and corn field. Data are represented as mean ± SD.

Fig. 5.6 Relationship between soil surface CO$_2$ flux (RHs and RH) and soil temperature from 2013 to 2014 (Corn). The line indicates an exponential regression of RHs and dot-dash line indicates an exponential regression of RH. Data are represented as mean ± SD.
Seasonal variation of soil respiration (RS) in managed grassland (2008-2012) and corn field (2013-2014) is shown in Fig. 5.7. The RS had a similar pattern with RH with the soil CO$_2$ flux increasing with the increase of soil temperature. The seasonal dynamics of RS were positively related to soil temperature in both managed grassland and corn field (Fig. 5.8). The Q$_{10}$ of RS was higher under managed grassland (3.0) than under corn field (1.9). In contrast with the result of RH, where annual RH increased by about 28% after the conversion to corn field, the annual average RS decreased by 20% two years after conversion to corn field. The annual average RS was 10.8 ± 1.0 Mg C ha$^{-1}$·yr$^{-1}$ in managed grassland and 8.6 ± 0.0 Mg C ha$^{-1}$·yr$^{-1}$ in corn field (Table 5.2). The contribution of annual RR to RS was 44% in in managed grassland and 6% in the corn field (Table 5.3).

Fig. 5.7 Seasonal variation in soil surface CO$_2$ flux (RS) in managed grassland (2008 to 2012) and corn field (2013 to 2014). Data are represented as mean ± SD.
Fig. 5.8 Relationship between soil surface CO₂ flux of RS and soil temperature in the managed grassland (2008 to 2012) and corn field (2013 to 2014). The line indicates an exponential regression in managed grassland and dot-dash line indicates an exponential regression in corn field. Data are represented as mean ± SD.

Table 5.2 Parameters of the exponential relationship between heterotrophic respiration (RHs and RH), heterotrophic respiration of manure (RHm) and soil respiration (RS) with soil temperature and annual average.

<table>
<thead>
<tr>
<th></th>
<th>R²</th>
<th>P value</th>
<th>Q₁₀</th>
<th>Average ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grassland</td>
<td>RHs</td>
<td>0.62</td>
<td>&lt;0.01</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>RH</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>RHm</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>RS</td>
<td>0.76</td>
<td>&lt;0.01</td>
<td>3.0</td>
</tr>
<tr>
<td>Corn</td>
<td>RHs</td>
<td>0.33</td>
<td>&lt;0.01</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>RH</td>
<td>0.34</td>
<td>&lt;0.01</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>RHm</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>RS</td>
<td>0.53</td>
<td>&lt;0.01</td>
<td>1.9</td>
</tr>
</tbody>
</table>

RH in managed grassland is the sum between RHs and RHm (Table 4.4).

Table 5.3 The ratio of heterotrophic respiration (RH) to ecosystem respiration (RE), root respiration (RR) to soil respiration (RR), autotrophic respiration (RA) to ecosystem respiration (RE), heterotrophic respiration (RH) to soil respiration (RS) and heterotrophic respiration of manure (RHm) to heterotrophic respiration (RH) in managed grassland and corn field.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Grassland</td>
<td>0.42</td>
<td>0.58</td>
<td>0.44</td>
<td>0.56</td>
<td>0.20</td>
</tr>
<tr>
<td>Corn</td>
<td>0.61</td>
<td>0.39</td>
<td>0.06</td>
<td>0.94</td>
<td>0.31</td>
</tr>
</tbody>
</table>
5.2.4 Plant biomass

The aboveground and belowground biomass were significantly influenced by land use. The annual harvest in corn field was significantly greater than in managed grassland. The annual harvest was $3.5 \pm 1.0$ and $7.7 \pm 0.9$ Mg C ha$^{-1} \cdot$yr$^{-1}$ in the managed grassland and corn field, respectively (Table 5.4). The annual ANPP in corn field was twice greater than in managed grassland. The annual ANPP ranged from 2.3 to $4.7$ Mg C ha$^{-1} \cdot$yr$^{-1}$ in the managed grassland and $8.9$ to $11.5$ Mg C ha$^{-1} \cdot$yr$^{-1}$ in corn field (Table 5.4). The annual average of BNPPes in managed grassland was greater than in corn field. The annual BNPPes in managed grassland ranged from $5.0$ and $4.7$ Mg C ha$^{-1} \cdot$yr$^{-1}$ in 2011 and 2012, respectively. In corn field, there was no significant difference between BNPPes ($0.7 \pm 0.4$ Mg C ha$^{-1} \cdot$yr$^{-1}$) and BNPP by biometric method ($1.0 \pm 0.0$ Mg C ha$^{-1} \cdot$yr$^{-1}$). The annual NPPes values in managed grassland were $9.5$ and $8.2$ Mg C ha$^{-1} \cdot$yr$^{-1}$ in 2011 and 2012, respectively (Table 5.4). In corn field, the annual NPPes in 2014 was greater than that NPPes in 2013. The annual NPPes was $9.3$ and $12.5$ Mg C ha$^{-1} \cdot$yr$^{-1}$ in 2013 and 2014, respectively (Table 5.4) During the conversion of managed grassland to corn field in 2012, there was no harvest in G3 period. Therefore, NPPes of G3 in 2012 is regarded as the residue ($2.1$ Mg C ha$^{-1}$) of managed grassland that will decompose in the next year. In corn field, the residue is the sum of stubble and root biomass. The residue of corn field was $1.0$ and $1.6$ Mg C ha$^{-1} \cdot$yr$^{-1}$ in 2013 and 2014, respectively.
Table 5.4 Annual ANPP, BNPP estimate ($\text{BNPP}_{\text{est}}$), BNPP biometric ($\text{BNPP}_{\text{bio}}$), and net primary production ($\text{NPP}_{\text{est}}$) in the managed grassland (2011 to 2012) and corn field (2013 to 2014) (Mg C ha$^{-1}$ yr$^{-1}$).

<table>
<thead>
<tr>
<th>Year</th>
<th>ANPP</th>
<th>$\text{BNPP}_{\text{est}}$</th>
<th>$\text{BNPP}_{\text{bio}}$</th>
<th>$\text{NPP}_{\text{est}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grassland</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2011</td>
<td>4.5 (0.8)</td>
<td>5.0</td>
<td>-</td>
<td>9.5</td>
</tr>
<tr>
<td>2012</td>
<td>3.5 (0.4)</td>
<td>4.7</td>
<td>-1.8 (2.6)*</td>
<td>8.2</td>
</tr>
<tr>
<td>Corn field</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2013</td>
<td>8.9 (1.9)</td>
<td>1.2</td>
<td>1.0 (0.2)</td>
<td>9.3</td>
</tr>
<tr>
<td>2014</td>
<td>11.5 (1.6)</td>
<td>0.6</td>
<td>1.0 (0.4)</td>
<td>12.5</td>
</tr>
</tbody>
</table>

ANPP and BNPP represent means $\pm$ (uncertainties/100 $\times$ means).  
* Annual BNPP in 2012 showed the sum of BNPP in NG, G1 and G2 periods.

### 5.2.5 Soil organic carbon

Soil carbon storage in managed grassland (2008 to 2012) and corn field (2013 to 2014) at 0–5, 5–10, 10–20 and 20–30 cm depth of soil is shown in table 5.5. At 0-30 cm depth interval the average total carbon soil storage in managed grassland was equal $155.4 \pm 8.1$ Mg C ha$^{-1}$, with values varying from 151.1 to 169.4 Mg C ha$^{-1}$ (Fig. 5.9). The average total carbon soil stock at the 0-30 cm depth interval in the corn field was equal $166.3 \pm 11.1$ Mg C ha$^{-1}$, with values varying from 158.5 to 174.2 Mg C ha$^{-1}$.

![Fig. 5.9 Annual soil carbon storage at 0 to 30 cm depth of soil from 2007 to 2014. Data are represented as mean ± SD.](image-url)
Table 5.5  Carbon soil storage in managed grassland (2008 to 2012) and corn field (2013 to 2014) at 0–5, 5–10, 10–20 and 20–30 cm depth of soil in manure plot at 10 and 20 m distance from the EC tower at four direction (south, west, north and east).

<table>
<thead>
<tr>
<th>Soil depth (cm)</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
<th>2012</th>
<th>2013</th>
<th>2014</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–5</td>
<td>28.3</td>
<td>30.1</td>
<td>27.6</td>
<td>30.3</td>
<td>31.1</td>
<td>26.2</td>
<td>31.6</td>
</tr>
<tr>
<td>5–10</td>
<td>25.0</td>
<td>26.4</td>
<td>25.7</td>
<td>25.1</td>
<td>24.1</td>
<td>25.9</td>
<td>29.6</td>
</tr>
<tr>
<td>10–20</td>
<td>50.0</td>
<td>56.2</td>
<td>47.0</td>
<td>44.6</td>
<td>49.9</td>
<td>52.8</td>
<td>57.4</td>
</tr>
<tr>
<td>20–30</td>
<td>48.2</td>
<td>56.6</td>
<td>49.1</td>
<td>54.3</td>
<td>47.0</td>
<td>53.6</td>
<td>53.8</td>
</tr>
<tr>
<td>Total</td>
<td>151.5</td>
<td>169.4</td>
<td>149.4</td>
<td>154.4</td>
<td>152.1</td>
<td>158.5</td>
<td>172.5</td>
</tr>
</tbody>
</table>

5.2.6 NBP

Annual NBP values are shown in Table 5.6. The annual NBP in managed grassland was 0.7 ± 0.7 Mg C ha⁻¹·yr⁻¹, with values ranging from 0.3 to 1.8 Mg C ha⁻¹·yr⁻¹. The annual NBP in corn field was -2.0 ± 0.4 Mg C ha⁻¹·yr⁻¹, with values ranging from -1.7 to -2.3 Mg C ha⁻¹·yr⁻¹.
Table 5.6 Annual net ecosystem production (NEP), gross primary production (GPP), ecosystem respiration (RE), heterotrophic respiration (RH), soil respiration (RS), autotrophic respiration (RA), root respiration (RR), harvest and net biome production (NBP) in managed grassland (2008-2012) and corn field (2013-2014) in manure (M) plot (Mg C ha$^{-1}$·yr$^{-1}$).

<table>
<thead>
<tr>
<th>Plot</th>
<th>Year</th>
<th>NEP</th>
<th>GPP</th>
<th>RE</th>
<th>RH</th>
<th>RS</th>
<th>RA</th>
<th>RR</th>
<th>Harvest</th>
<th>NBP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grassland</td>
<td>2008</td>
<td>2.6</td>
<td>16.9</td>
<td>14.3</td>
<td>-</td>
<td>9.6</td>
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<td>4.3</td>
<td>0.4</td>
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<tr>
<td></td>
<td>2009</td>
<td>2.7</td>
<td>15.9</td>
<td>13.3</td>
<td>-</td>
<td>10.1</td>
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<td>-</td>
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<tr>
<td></td>
<td>2010</td>
<td>1.7</td>
<td>17.1</td>
<td>15.3</td>
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<td>1.3</td>
</tr>
<tr>
<td></td>
<td>2011</td>
<td>3.1</td>
<td>19.0</td>
<td>15.9</td>
<td>6.2</td>
<td>10.8</td>
<td>9.5</td>
<td>4.5</td>
<td>4.7</td>
<td>0.4</td>
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<tr>
<td></td>
<td>2012</td>
<td>1.8</td>
<td>17.0</td>
<td>15.0</td>
<td>6.3</td>
<td>12.0</td>
<td>8.7</td>
<td>5.7</td>
<td>2.8</td>
<td>0.3</td>
</tr>
<tr>
<td>Corn field</td>
<td>2013</td>
<td>1.2</td>
<td>14.8</td>
<td>13.6</td>
<td>8.1</td>
<td>8.6</td>
<td>5.5</td>
<td>0.5</td>
<td>8.3</td>
<td>-1.7</td>
</tr>
<tr>
<td></td>
<td>2014</td>
<td>4.4</td>
<td>17.3</td>
<td>12.9</td>
<td>8.1</td>
<td>8.7</td>
<td>4.9</td>
<td>0.6</td>
<td>10.9</td>
<td>-2.3</td>
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Result of ANOVA

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<th></th>
<th>d.f.</th>
<th>F</th>
<th>p</th>
<th>F</th>
<th>p</th>
<th>F</th>
<th>p</th>
<th>F</th>
<th>p</th>
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<th>p</th>
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<td>0.3</td>
<td>3.8</td>
<td>0.1</td>
<td>1369</td>
<td>&lt;0.01</td>
<td>8.5</td>
<td>&lt;0.05</td>
<td>60.8</td>
<td>&lt;0.05</td>
<td>57.1</td>
<td>&lt;0.05</td>
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</table>
5.3 Discussion

5.3.1 Effect of land use on RH and RS

The accumulation of soil organic C is the result of a delicate balance between C fixation and microbial decay of senescent vegetation (mainly root mass and residues). Conversion to corn field changed the dynamic equilibrium between inputs and outputs established in the previous five years of managed grassland. The conversion from managed grassland to corn field caused an increase in soil C emission. The RH in corn field \((8.1 \pm 0.0 \text{ Mg C ha}^{-1}\text{.yr}^{-1})\) was significantly greater than in managed grassland \((6.4 \pm 0.0 \text{ Mg C ha}^{-1}\text{.yr}^{-1})\) (Table 5.2). The contribution of RH to RS in corn field (94%) was greater than that in managed grassland (56%) (Table 5.3). The increase of RH after the conversion from managed grassland to corn field was mainly because of tillage-induced aeration and stronger soil aggregation (Paustian et al., 1997). Tillage associated with soil mixing and inversion, which was carried out in corn field, promotes breakdown of soil aggregates and structure and expose physically protected soil organic carbon to microbial decomposition (Schjoning and Rsmussen, 2000). Tillage also exposes surface soils to the freeze–thaw and wet–dry cycles which accelerates breakdown of macro aggregates (Six et al., 2004). No tillage in managed grassland enhances soil aggregate stability which slows the decomposition of soil organic matter by providing protection within soil aggregates (Ussiri and Lal, 2009).

The temperature dependency of heterotrophic and soil respiration, \(Q_{10}\), is an important parameter for predicting RH and RS, at a given temperature and it changes with land use, micro-environment and ecosystem type (Pavelka et al., 2007; Peng et al., 2009). Wang et al. (2008) reported \(Q_{10}\) values ranging from 1.2 to 3.2 for a forest, from 1.3 to 3.5 for a meadow, and from 1.2 to 3.7 for a farm. Many studies
have shown that $Q_{10}$ changes in response to change in soil temperature, the spatial and temporal scales of the system, and substrate availability (Wu and Qi, 2001; Fierer et al., 2005; Gaumont-Guay et al., 2006; Wang et al., 2006; Almagro et al., 2009). When $Q_{10}$ is close to or less than 1, it means that soil respiration is not sensitive to temperature, whereas it is extremely sensitive at values greater than 2 (Xu and Qi, 2001; Pavelka et al., 2007). In our study, $Q_{10}$ of RS in managed grassland was 3.0 greater than 2, while in corn field it was 1.9, slightly lower than 2. The $Q_{10}$ of RH (RHs: 1.8 and RH: 1.6) in corn field was slightly lower than $Q_{10}$ of RH (RHs: 1.9) in managed grassland (Table 5.2). The $Q_{10}$ of RHs, RH and RS in both managed grassland and corn field was either slightly lower or greater than 2, which means RHs, RH and RS was sensitive to soil temperature in both land uses.

Land use change and management practices strongly influence global C dynamics, especially in terms of their effects on RS (Zhang et al., 2012). The vegetation structure and species composition, both of which change in response to changes in land use, also strongly influence the C allocation pattern (Wang et al., 2006). In this study, RS differed among the two land use types. RS under corn field was significantly lower than under managed grassland after the conversion. Wang et al. (2014) found that RS averaged 8.8 Mg C ha$^{-1}$·yr$^{-1}$ under 15 years of restored grassland which was higher compared to 4.7 Mg C ha$^{-1}$·yr$^{-1}$ under cropland (wheat and maize), because the grassland had a significantly greater root biomass and return of plant biomass. Soil respiration originates mainly from root and microbial activity, and this makes RR an important component of RS on the global carbon balance (Hogberg et al., 2001; Jia et al., 2006). The contribution of RR to RS varies among vegetation types, vegetation ages, species composition, soil types, season of the year, and environmental conditions (Hanson et al., 2000; Raich et al., 2002; Lee et al.,
2003; Tang et al. 2005; Gong et al., 2012). Yazaki et al. (2004) reported that contribution of RR to RS ranged from 22-53% in temperate grassland (*M. sinesis*); Shimizu et al. (2009) reported a range of 49-64% in managed grassland; and Jans et al. (2010) reported 38% in corn field. In our study, the contribution of RR to RS in managed grassland was seven times greater than in corn field (Table 5.3). Although the soil organic matter decomposition (RH) in corn field was greater than managed grassland due to management practices such as tillage event and manure application, the conversion of managed grassland to corn field would significantly decrease total CO₂ fluxes (RS) from the soil to the atmosphere due to the reduction of root biomass.

5.3.2 Impact of land use change on C sequestration

The NEP of managed grassland exhibited variability over time. Inter-annual variability in NEP is common in grassland ecosystems (Gilmanov et al., 2010; Meyers, 2001, Aires et al., 2008, Zenone et al., 2013). Overall, grasslands tend to be C sinks during years with favorable precipitation (Zhang et al., 2010). The result of this study showed that the managed grassland sequestered C, and the C dynamics were driven by environmental factors such as precipitation, air temperature and PAR.

The NEP showed a response after herbicide application when the field was converted from grassland to corn field (Fig. 5.3). Large difference between annual NEP in 2013 and 2014 was potentially driven by NPP. The NPP_{est} in 2014 was 34% greater than in 2013 (Table 5.4), which was affected by the amount of GPP. The GPP was positively correlated with NPP. Another factor that potentially drove NEP was RE. The annual RE in 2013 was slightly greater than RE in 2014 (Table 5.3). Thus higher RE in 2013 was probably due to the large CO₂ flux from the decomposition of grass biomass killed during the conversion in the previous year. Zenone et al. (2011)
reported that conversion of perennial grassland and soybean to an annual cropping system invoked a large emission of CO$_2$ during the first year of conversion, mainly due to the decomposition of dead aboveground biomass present after the herbicide application and decomposition of root.

Manure application rate in the corn field was five times more than in managed grassland. As a consequence of the large amount of fresh manure in the corn field, the average annual manure decomposition in the corn field (2.5 ± 0.0 Mg C ha$^{-1}$·yr$^{-1}$) was two times greater than in managed grassland (1.3 ± 0.1 Mg C ha$^{-1}$·yr$^{-1}$) (Table 5.2). The contribution of RHm to RH in corn field (31%) was greater than that in managed grassland (20%) (Table 5.3). The RHm accounted for 40 and 52% of the total manure C applied in managed grassland and in corn field, respectively. The conversion from managed grassland to corn field increased soil C by 7%. These results were probably due to higher amount of C input into the soil from the cattle manure in the corn field. Liu et al. (2008) measure long-term (1979 to 2008) effect of manure application on soil organic C pools in dryland farming in Northwest China and found that the soil organic C from 0 to 60 cm depth of soil was increased by 41.3% in farmyard manure with supplementary chemical fertilizer treatments.

Accounting for C import and C export, the NBP of managed grassland during five years remained positive. The average NBP of managed grassland was 0.8 ± 0.7 Mg C ha$^{-1}$·yr$^{-1}$, which was similar to values reported in other grassland ecosystem. Ammann et al. (2007) found positive value for NBP (1.5 Mg C ha$^{-1}$·yr$^{-1}$) in grasslands in Switzerland with manure and fertilizer treatment. However, the conversion from managed grassland to corn field decreased the NBP into negative (-2.0 ± 0.4 Mg C ha$^{-1}$·yr$^{-1}$), although NEP was positive during two years study period and C import was five times more than in managed grassland. The net C loss from corn field was mainly
caused by the export from biomass. These result are similar to those reported by Jans et al. (2010) who measured the NBP of corn field in the Netherlands with manure treatment (0.51 Mg C ha$^{-1}$) and found that corn field is a moderate source of C.

5.4 Conclusion

The C budget after conversion from managed grassland (2008 to 2012) to corn field (2013 to 2014) with composed cattle manure and supplementary chemical fertilizer treatment was estimated. The conversion of managed grassland to corn field significantly decreased RS due to the reduction of RR. Annual RH after the conversion from managed grassland to corn field significantly increased due to increase of RHm, which may be ascribed to tillage in corn field. Although the amount of manure C applied in corn field was 2.5 times greater than that in managed grassland, the conversion from managed grassland to corn field caused the ecosystem to be C source.
Chapter 6. General discussion

6.1 Effect of management practices on C budget

Management practices affected C budget in the ecosystems. Daily?? GPP quickly decreased after the harvesting whereas the herbicide application took several days to decrease GPP to the same level as that after harvest (Fig. 5.2 and Fig. 5.3). Reduction in NEP was caused by decrease of GPP, which was affected by herbicide application, or increase of RE due to the effect of tillage events or a combination of both. In this study, decrease in living vegetation decreased GPP which contributed to the large reductions in NEP shortly after herbicide application and harvest. The RE decreased with decrease of RA, but RE also increased with increase of RH due to addition of new labile C sources. In this study, herbicide application and harvest did not change RE greatly (Fig. 5.3 and Fig. 5.4). Eugster et al. (2010) observed the effect of management practices on ecosystem respiration by comparing the respiration fluxes during 7, 14 and 28 days after the management event and found that the greatest changes in RE was during the 7-days following tillage and irrigation events. However during 28 days after management practices, the effect of herbicide applications on RE was lower than the effect of irrigation. Managed grassland began to uptake CO₂ (positive NEP) about two weeks after the first harvest (Fig. 5.4). However, the CO₂ uptake after the second harvest was lower than after first harvest, and third harvest was further lower than first and second harvest. This was due to decrease of NEP from G1 to G3. Similar results were found by Schmitt et al. (2010) who reported that the grassland took an average of 14 days after the first harvest to recover and become a net sink for CO₂. Hussein et al. (2011) measured NEP of extensively managed grassland in Germany and found that the increase of annual frequency of harvests decreased CO₂ sink potential of grassland.
Fresh manure was added to managed grassland and corn field. The applications of manure into the field were not followed by any immediate noticeable changes to NEP, GPP and RE. Eugster et al. (2010) also found that respiration did not generally increase following organic fertilizer application. The continuous manure application from managed grassland (2008 to 2012) to corn field (2013 to 2014) was slightly increased soil C storage by 7%. It was due to the amount of manure C applied in corn field \((4.9 \pm 0.8 \text{ Mg C ha}^{-1}\cdot\text{yr}^{-1})\) was 2.5 times greater than that in managed grassland \((1.9 \pm 0.4 \text{ Mg C ha}^{-1}\cdot\text{yr}^{-1})\).

Usually increase of soil CO\(_2\) emission following tillage events is expected. There are several reports that CO\(_2\) emissions were proportional to the intensity of the tillage events; more specifically tillage depth and post tillage surface roughness (Reicosky, 1997; Reicosky and Lindstrom, 1993; Reicosky et al., 2005). Tillage, similar to the effect of harvest events, led to a rapid decrease in NEP. The decrease in NEP could be due to either reduction in GPP or an increase in RE. Since there was very little photosynthesis vegetation in the field at the time of tillage, the cause of decrease of NEP can be attributed to increase of RE. As winter approached, air temperature decreased causing photosynthesis to slow down and eventually stop, marked by reduction in NEP, GPP and RE. Therefore, tillage at low temperature is recommend to reduce soil CO\(_2\) emission caused by tillage.

### 6.2 Effect of land use conversion on C budget

In managed grassland the BNPP\(_{\text{est}}\) value was more reasonable than BNPP\(_{\text{bio}}\). There was no significant difference between annual BNPP\(_{\text{est}}\) and BNPP\(_{\text{bio}}\) in corn field. The BNPP\(_{\text{est}}\) in managed grassland was greater than in corn field due to the vegetation type (Table 5.4). Although the RH was higher in corn field than in
managed grassland due to the higher RHm,, there was no significant difference in RE between managed grassland and corn field (Table 5.3). This result is probably due to the decrease of RR after the conversion to corn field, which is supported by the decreasing of RS. The overall effect of conversion seems to be most strongly related to management practices and the type of the vegetation.

6.3 Conclusion

The results of this study have shown the effect of management practices on the different components of C cycle and their influence on the C budget in managed grassland and corn field. Management practices such as harvest, pesticide addition, tillage and manure application significantly influence the C budget. Manure application with supplementary chemical fertilizer increased biomass production. However, manure application increased annual RE due to increase of RHm. The RE was also influenced by tillage event, rapidly increasing following the reduction of NEP. Harvest and herbicide applications decreased GPP while RE remained constant. In case of managed grassland, long-term manure application enhanced annual NBP, but not for the corn field where NBP was shown as C source due to high C export from the ecosystem.
Summary

Carbon dioxide ($\text{CO}_2$) exchange between terrestrial ecosystem and the atmosphere results from photosynthesis and autotrophic and heterotrophic respirations. Photosynthesis assimilates atmospheric $\text{CO}_2$ to the plant body, which is the main natural carbon (C) import into the ecosystem, called as gross primary production (GPP). Autotrophic and heterotrophic respirations emit $\text{CO}_2$ from plant body and dead organic matter including soil organic matter to the atmosphere, respectively, which are the main natural C export from the ecosystem, called as ecosystem respiration (RE). The difference between GPP and RE is net ecosystem production (NEP), and is directly measured by eddy covariance method. The NEP represents the amount of C used to produce new biomass in the ecosystem. Therefore, NEP is also described as the difference between net primary production (NPP) and heterotrophic respiration (RH), which are measured by biometric method and chamber method, respectively. The RH is often measured in the root exclusion plot. Root respiration (RR) is estimated as the difference between soil respiration (RS) measured in root intact plot and RH. In agricultural ecosystem, management practices influence the ecosystem C budget. Manure application is the main anthropogenic import of organic C into the ecosystem, and harvest is the main anthropogenic export of organic C from the ecosystem. Therefore, C budget in agricultural ecosystem is estimated as $\text{NEP + manure application - harvest}$, which is called as net biome production (NBP). In this study, the combination of eddy covariance, biometric and chamber methods was used to evaluate and compare the effect of manure application on the C budget in managed grassland and corn field.
1. Effect of manure application on the C budget of managed grassland

The C budget of a managed grassland in Shin-Hidaka, Hokkaido, Japan was estimated for five years (2008-2012). Chemical fertilizer was applied to fertilizer (F) plot at a rate of 79 ± 20 kg N ha\(^{-1}\)·yr\(^{-1}\). In the manure (M) plot, dairy cattle manure was applied at a rate of 10 Mg fresh matter ha\(^{-1}\)·yr\(^{-1}\) (1923 ± 407 kg C ha\(^{-1}\)·yr\(^{-1}\), 159 ± 68 kg N ha\(^{-1}\)·yr\(^{-1}\)). There was no significant difference in seasonal GPP and harvest between the treatment plots, indicating that both fertilizer and manure can increase the biomass production. Annual NEP and RE were significantly different between the treatment plots. The difference in RE, and between M and F plots is probably due heterotrophic respiration of manure (RHm), which ranged from 1.2 to 1.3 Mg C ha\(^{-1}\)·yr\(^{-1}\). Average annual RHm was 1.3 ± 0.1 Mg C ha\(^{-1}\)·yr\(^{-1}\), and accounted for 40% of the total amount of applied manure C. The annual NBP in the M plot (from 0.5 to 1.5 Mg C ha\(^{-1}\)·yr\(^{-1}\)) was significantly higher than in the F plot (-1.1 to 0.7 Mg C ha\(^{-1}\)·yr\(^{-1}\)). The long-term effect of manure application combined with chemical fertilizer did not reduce grass production compared with chemical fertilizer only; however, manure application decreased the NEP through manure decomposition, and long-term manure application enhanced the NBP.

2. Land use change effect on C budget

The knowledge on the effect of land use change on C budget is limited. This study compared the effect of manure application in managed grassland and corn field. The manure applied managed grassland was converted into corn field in 2013 and C budget was measured for two years. 50 Mg fresh matter ha\(^{-1}\)·yr\(^{-1}\) (4864 ± 823 kg C ha\(^{-1}\)·yr\(^{-1}\), 268 ± 108 kg N ha\(^{-1}\)·yr\(^{-1}\)) of manure was applied to the corn field. Annual GPP
and RE slightly decreased after the conversion from managed grassland to corn field, while NEP slightly increased. Annual harvest in corn field was three times greater than that in managed grassland. The conversion to corn field significantly decreased RS due to the reduction of RR. Annual RH significantly increased due to increase of RHm after the conversion to corn field, which may be attributed to tillage in corn field. Annual RHm in corn field was $2.5 \pm 0.0 \text{ Mg C ha}^{-1} \cdot \text{yr}^{-1}$ which accounted for 52% of manure C application rate. Consequently, annual NBP in corn field decreased to $-2.0 \pm 0.4 \text{ Mg C ha}^{-1} \cdot \text{yr}^{-1}$. Although the amount of manure C applied in corn field ($4.9 \pm 0.8 \text{ Mg C ha}^{-1} \cdot \text{yr}^{-1}$) was 2.5 times greater than that in managed grassland ($1.9 \pm 0.4 \text{ Mg C ha}^{-1} \cdot \text{yr}^{-1}$), the conversion from managed grassland to corn field led the ecosystem to be C source.
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