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1	Soil profile carbon and nutrient stocks under long-term conventional and
2	organic crop and alfalfa-crop rotations and re-established grassland
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2 Soil carbon stocks are useful indicators of both C sequestration capacity and sustainability 3 of agricultural systems. Yet, most investigations have only studied the effects of 4 agricultural management on soil carbon in surface layers (< 0.3 m). Current soil organic 5 carbon (SOC), total soil nitrogen (TN) and plant available phosphorus (P_{Olsen}) to a depth of 6 1.2 m was measured at two long-term (9 and 18 years) farming systems experiments in 7 southern Manitoba, Canada. Both experiments compared an annual-crop rotation, an 8 alfalfa (Medicago sativa L.)/crop rotation and re-established perennial grassland. At one 9 site the two cropping systems were managed conventionally as well as in adherence to 10 organic farming guidelines, but without manure additions. Due to higher net primary 11 productivity and higher carbon inputs, particularly below ground, SOC stocks (0-120 cm) were 21-65 t C ha⁻¹ higher under the re-established grassland than cropping systems at the 12 13 clay soil site after 18 years, but not at the site with sandy loam soil after 9 years. On the 14 clay soil, 30-40% of the additional C in the soil profile under the re-established grassland 15 was found below 30 cm indicating the capacity of deep plant roots to sequester C in the 16 sub-soil. Using alfalfa cut for hay in crop rotations did not increase SOC or N stocks 17 compared to annual crop rotations, but plant-available P concentrations were depleted, especially under organic management. SOC was 25-30 t C ha⁻¹ lower under organic than 18 conventionally managed cropping systems, due to lower inputs of plant C (0.8 t C ha⁻¹ yr⁻¹) 19 20 over the life of the experiment. This highlights that without additional C inputs organic 21 management can reduce SOC compared to conventional cropping systems unless C inputs 22 are maintained which may require manure or compost additions.

23

24 Keywords: soil health, prairie, sequestration, alfalfa, phosphorus, nitrogen, organic,

25 agriculture

1 1. Introduction

2 Soil organic carbon (SOC) and nutrient stocks are important indicators of long-term 3 agricultural production system sustainability. In particular, many attributes of soil quality 4 that affect agricultural productivity and ecosystem function are influenced by soil organic 5 carbon status, such as soil structure and physical fertility, microbial activity, nutrient 6 availability and cycling (Lal, 1998). In addition, management induced changes in soil 7 carbon status under different agricultural uses have implications for soil C stocks and the 8 ability to sequester carbon or mitigate CO₂ emissions in agricultural production systems 9 (Johnson *et al.*, 2007). Soil carbon is also often associated with other major nutrients, 10 especially nitrogen and phosphorus, and hence influence cycling and availability of 11 nutrients for crop and pasture production.

12

13 Perennial-based agricultural systems are generally beneficial for maintaining or building 14 soil carbon. Various studies around the world have shown that perennial grass-legume 15 pastures, in particular, are effective for increasing soil C compared to annual cropping 16 systems (Dalal et al., 1995; Franzluebbers et al., 2000; Gentile et al., 2005; Franzluebbers 17 and Stuedemann, 2009). The accumulation of C and N is enhanced when significant 18 proportions of legumes are present (Robles and Burke, 1997). Land converted from 19 cultivated crop production into revegetated grassland in the Conservation Reserve Program 20 in the US, have shown significant increases in SOC, but the size and speed of these 21 changes seems to be affected by soil characteristics (e.g. soil texture, P concentration) 22 (Burke et al., 1995; Reeder et al., 1998; Bowman and Anderson, 2002). Few studies have 23 reported changes in SOC after restoring native perennial grasslands on the Canadian 24 prairies, and hence the potential for sequestering soil C by changing current land use from 25 agricultural production to native grasslands has not been established experimentally. In the 26 past most studies investigating the effects of long-term agricultural management on soil

1

- 2 cropping frequency or intensity (Janzen *et al.*, 1998; McConkey *et al.*, 2003).
- 3

Perennial forage legumes grown in rotation with crops appear to be less effective at 4 5 increasing SOC than grass-based systems. On the Canadian prairies, alfalfa (Medicago 6 sativa L.) hay crops in cereal rotations have increased surface (top 0.3 m) SOC compared 7 to fallow-wheat systems, but not compared to continuous crop rotations (Janzen et al., 8 1998). In Ontario, Canada, after 35 years alfalfa-maize (Zea mays L.)-oat (Avena sativa L.) 9 rotation had higher SOC in the top 0.7 m than maize monoculture, but less than a mown 10 perennial bluegrass pasture (Poa pratensis L.) (Gregorich et al., 2001). In Australia, alfalfa 11 grown in rotation with cereal crops, has not increased surface (0-0.1 m) SOC compared to 12 annual cropping systems, and significantly less than grass-legume pastures (Dalal *et al.*, 13 1995; Whitbread et al., 2000).

14

15 Organic production can also increase SOC compared with conventional systems (Johnson 16 et al., 2007). A number of practices associated with organic management are likely to 17 increase soil C sequestration (e.g. surface mulching, manure application, reducing fallow 18 length, cover cropping, green manuring, crop rotation). In particular, manure and compost 19 application appears to be an important driver of higher soil carbon stocks in organic 20 systems (Pimentel et al., 2005; Fließbach et al., 2007). The gains observed from organic 21 management are often significantly reduced when regular manure applications are omitted 22 (Clark et al., 1998; Pimentel et al., 2005) or additional gains are minimal due to omission 23 of chemical weed and pest control (Fließbach et al., 2007). However, gains in soil C in 24 organic systems have been attributed to the use of legume green manure crops (Pimentel et 25 al., 2005) and by changing to organic pest and disease control measures (Birkhofer et al., 26 2008).

1

2 Most evaluations of agricultural management practices on soil organic carbon have 3 focussed on the soil surface (top 0.30 m) and relatively little is known about the impact of 4 agricultural management on soil carbon or nutrient stocks throughout the soil profile 5 (Thorburn et al., 2012). Predicting changes in soil C stocks of agricultural systems without 6 accounting for changes in soil C in deeper soil layers can be problematic (Baker et al., 7 2007). Considering deeper soil layers is also particularly important when comparing 8 systems that include annual and perennial plants, because of the greater allocation of roots 9 in deep soil layers in perennials. In one of a few studies on long-term effects of annual vs. 10 perennial systems on subsoil SOC, Gentile et al. (2005) showed that a system in southern 11 Uruguay, including a perennial (alfalfa) in rotation had more SOC between 30 and 60 cm 12 than annual grain-only rotations.

13

14 From the above, we can see that the impact of perennial forages and organic farming 15 systems on SOC is unclear, especially when considering SOC stocks to the depth of the 16 root zone. Current soil carbon and nutrient stocks throughout the soil profile (to 1.2 m) 17 were measured under two long-term farming systems experiments in southern Manitoba, 18 Canada. Rates of change in soil carbon and nutrient stocks could not be calculated as soil 19 samples taken at the initiation were not available for analysis; hence comparisons are made 20 assuming all plots were approximately equal in soil C and nutrients at the initiation of the 21 treatments. At these sites, an annual crop rotation, an alfalfa-crop rotation and restored 22 native perennial grassland had been subjected to consistent management for the past 9 and 23 18 years, respectively. At one of the sites, the two crop rotations were also subject to 24 organic and conventional management. The following questions were explored: 25 1. How much more soil carbon can be stored under re-established perennial

- 26
- grasslands compared to cropping systems?

Do rotations that include perennial forages (e.g. alfalfa) maintain higher levels of
 soil carbon and nutrients than crop rotations based solely on annual crops?
 Does organic versus conventional management affect levels of soil carbon and
 nutrients?

5 **2. Methods**

6 The two long-term farming systems experiments sampled in this study were located in the 7 Red River valley of southern Manitoba, Canada at the University of Manitoba Glenlea 8 Research Station (49°38'25"N, 97° 8'28"W, 230 m above sea level) and the Ian N. Morrison Research Farm at Carman (49°29'48"N, 98° 2'26"W, 267 m above sea level). 9 10 The climate in the region is mid-latitude moist continental, having very cold winters and 11 warm summers; annual mean maximum temperature is 8.3 °C and annual mean minimum 12 temperature is -3.1 °C (Environment Canada, 2012). The frost-free crop growing period 13 averages 122 days, beginning last week of May and ending last week of September. 14 January is the coldest month (-12.7 °C mean maximum and -22.8 °C mean minimum 15 temperature) and July the warmest month (25.8 °C mean maximum and 13.3 °C mean 16 minimum temperature) (Environment Canada, 2012). Total sunshine in July averages 304 17 hours over the month. Total precipitation averages 514 mm with 415 mm occurring as rain, 18 and 100 mm as snow. The wettest months are June to August which receive 235 mm on 19 average, while November to March are the driest months receiving average monthly rain of 20 less than 25 mm. The soil types at the two sites differ substantially (Table 1); the Glenlea 21 site occurs on the Scanterbury and Hoddinott series, two very similar heavy clay Humic 22 Vertisol soils, and Carman on Hochfeld Series, a sandy loam textured soil classified as an 23 Orthic Black Chernozem (Soil Classification Working Group, 1998). 24

25 INSERT TABLE 1 HERE

1 2.1 Glenlea farming systems experiment

2 This long-term cropping system experiment commenced in 1992 and had undergone 18 3 years comparing four crop rotations; annual-crop and alfalfa/crop rotations managed with 4 both organic and conventional practices. During the first 12 years of the experiment (three 5 cycles of the rotations) the whole treatment area followed the crop sequence outlined in Figure 1. The thirteenth year (2004) of each rotation was phased so that in each treatment 6 7 all crops were grown in the same year. The experiment is arranged in a completely 8 randomized design with three replicate plots of each treatment. In the four crop rotations 9 these replicates are distributed across two management blocks. Organic and conventional 10 blocks are separated by 15 m in order to reduce soil and pesticide movement between 11 plots, as well as pest and weed transfer.

12

13 This experiment was managed using tillage with crop residues incorporated into the soil 14 with a disc and a field cultivator prior to sowing each year. Organic plots were managed 15 with no external inputs of manures or other amendments. In conventionally-managed plots, 16 soil was tested for nutrient status in autumn of most years, and nitrogen fertilizer rates were based on soil test recommendations; average N fertiliser application was 100 kg N ha⁻¹ for 17 wheat after soybean and 75 kg N ha⁻¹ for wheat after alfalfa, 60 kg N ha⁻¹ for flax, 90 kg N 18 ha⁻¹ for oats. Nitrogen fertilizer was broadcast and incorporated prior to seeding (1992 to 19 20 2003). After 2003, N fertilizer was mid-row banded along with the seeding operation. Phosphorous fertilizer was applied at a basal rate of 20-25 kg P ha⁻¹ during the seeding 21 22 operation of crops. No other nutrients were required. Seeding rates were 250 viable seeds m^{-2} for all crops except alfalfa, which was seeded at 12 kg ha⁻¹. Both pre-sowing and in-23 24 crop herbicides were used in the conventional plots in all years. Fewer grass control 25 herbicides were required in the alfalfa-crop rotation compared with the annual-grain only 26 rotation. All grain crops were harvested for grain at maturity, except fababean (Vicia faba

L.) in the annual-organic rotation which was treated as a green manure crop. Alfalfa was
 cut for hay twice per year each growing season and the cut material removed from the
 plots.

4

5 INSERT FIG. 1 HERE

6

7 In addition to the cropping treatments, a re-established perennial grassland treatment was 8 established at the site in spring of 1993. This included a mix of indigenous warm- and 9 cool-season perennial grasses; northern wheatgrass (Elymus lanceolatus (Scribn. & J.G. 10 Sm.) Gould syn. Agropyron dasytachum), slender wheatgrass (Elymus trachycaulus (Link) 11 Gould ex Shinners), western wheatgrass (Pascopyrum smithii (Rydb.) A. Löve), Indian 12 grass (Sorghastrum nutans (L.) Nash), switch grass (Panicum virgatum L.) and big 13 bluestem (Andropogon gerardii Vitman). Native grassland plots were subjected to a spring 14 burn every 4-5 years (1996, 2000 and 2005), but have not been grazed or cut.

15 2.2 Carman farming systems experiment

This long-term cropping system experiment commenced in 2000 comparing two crop rotations; an annual-crop rotation and an alfalfa/crop rotation. Both four-year crop rotations were phased so that each crop was grown each year and had been through two cycles when soil was sampled (Figure 1). A bare fallow treatment (maintained with regular glyphosate applications) and a re-established native grassland treatment (sown in 2000) are also present at the site. The experiment is arranged in a randomized block design with three replicate plots of each treatment.

This experiment was managed in a complete no-till system using a low disturbance no-till
disc seeder, hence all crop residues remained on the soil surface and were not incorporated.
Row spacing, seeding and fertiliser rates, harvest and straw management used in this study
were typical of no-till producers in western Canada. Oat, flax and wheat were seeded at

130 kg ha⁻¹, canola was seeded at 8 kg ha⁻¹, and alfalfa was seeded at 12 kg ha⁻¹. Fertilisers 1 2 were applied to provide adequate N, P, K and S according to annual soil test 3 recommendations; the average application over each 4-year-cycle for the annual-crop rotation was 77 kg N yr⁻¹, 17.5 kg P yr⁻¹, 11 kg K yr⁻¹ and 23 kg S yr⁻¹ and for the alfalfa-4 crop rotation was 22 kg N yr⁻¹, 17.5 kg P yr⁻¹, 26 kg K yr⁻¹ and 15 kg S yr⁻¹. All plots were 5 sprayed with 3.01 ha⁻¹ glyphosate (360 g l⁻¹ formulation) prior to, or just after, seeding 6 each year. Post-emergent herbicides were applied in early to mid June to all crops except 7 8 alfalfa. All grain crops were harvested for grain at maturity, and alfalfa was cut for hay 9 twice each growing season and removed from the plots. Species sown in the re-established 10 native grassland were the same as those sown at Glenlea (described previously). Similar to 11 the management at Glenlea, this was burned once 5 years after sowing (i.e. 2005), but has 12 not been grazed or cut.

13 2.3 Estimates of carbon inputs from farming systems

Measurements of crop biomass, yield of grain or forage (alfalfa), and weed biomass were 14 15 taken from each of the farming systems throughout experiments. All plant biomass was 16 assumed to have 40% C content. This data was used to estimate the net primary 17 productivity (NPP) and total annual carbon input over the life of the experiments using 18 methods outlined by Bolinder et al. (2007). Annual plant C allocation coefficients to 19 agricultural product such as hay or grain (R_P) , post-harvest residues (R_S) , root tissue (R_R) and in extra-root material (R_E) for Canadian agro-ecosystems were used for wheat (R_P – 20 21 $0.322, R_{S} - 0.482, R_{R} - 0.118, R_{E} - 0.078)$, oats (R_P - 0.319, R_S - 0.283, R_R - 0.241, R_E - 0.078) 22 0.157), perennial forage legumes for alfalfa ($R_P - 0.571$, $R_S - 0$, $R_R - 0.26$, $R_E - 0.169$), 23 and grassland/pasture for the re-established grassland ($R_P - 0.233$, $R_S - 0$, $R_R - 0.465$, $R_E - 0.465$ 24 0.302). Coefficients were not provided for the broad-leaf grain legume fababean and 25 oilseed crops flax and canola; other data suggest these are similar across pulse and oilseeds 26 so coefficients for soybean were used ($R_P - 0.304$, $R_S - 0.455$, $R_R - 0.146$, $R_E - 0.095$)

1 (Gan et al., 2009). Harvest losses for grain and alfalfa forage were assumed to be 15% of 2 harvested yield and were added to the post-harvest residue pool (C_s) (Bolinder et al., 3 2007). Above-ground biomass production from the alfalfa at Carman was only recorded in 4 5 of the 9 growing seasons and for the re-established grassland was only measured in 8 of 5 the 17 years at Glenlea. Biomass production from the re-established grassland at Carman 6 was not measured. Annual biomass production was averaged and used to calculate NPP 7 over the full experimental period. In the grassland, all surface residues were maintained, 8 except the years where the grassland was burnt; no surface residue C inputs from these 9 years were included.

10 2.4 Soil sampling procedures

11 Five 4 cm diameter \times 120 cm soil cores were taken from each of the three replicated plots 12 that had been treated with the different farming systems (i.e. 15 cores per treatment per 13 site). Soil cores were only taken from one phase of each of the crop rotations in June, 2009. 14 These were chosen so that a cereal crop had been grown in each rotation in the previous 15 year. Hence, samples were taken in plots that in the previous year had grown oat at 16 Carman, oat in the organic plots at Glenlea, and wheat in the conventional plots at Glenlea. 17 Intact soil cores were extracted into clear polyethylene terephthalate sleeves using a 18 hydraulic soil coring rig (The Giddings Machine Co, Windsor, CO, USA) with a 4 cm 19 diameter tip, then sealed and stored at 4°C prior to processing.

20

Before each intact soil core was segmented into layers, the entire core was weighed, its length measured and inspected for evidence of compaction that may have occurred during its extraction. Approximately 15 % of cores were found to have compaction and this was usually evidenced by a higher than normal wet mass per unit length for the entire intact core. Using an average density for other cores with no evidence of compaction (i.e. 19.3 g soil cm⁻¹ depth at Glenlea and 21.5 g soil cm⁻¹ depth at Carman), the length of 'uncompacted' core was calculated, and the intervals used for each layer of the core
adjusted proportionally (e.g. from 15 cm to 14 cm). This was done to ensure a similar
mass of soil was allocated into each layer. This only influenced layer intervals and not any
calculation of soil bulk density in each layer, and hence the total soil mass in the profile
measured.

6

Cores were cut into layers and the length and mass of each layer recorded. Any root
material was removed by hand from the sample. Each layer was sub-sampled and dried (at
105°C) for gravimetric water content determination. This gravimetric water content was
then used to calculate the dry soil mass and bulk density in each layer. Air-filled porosity
(*E*) of soil was calculated with equation (1)

12
$$E = (1 - \rho / 2.65) - \theta$$
 (1)

13 where ρ is the measured bulk density (Mg m⁻³), $\theta = w\rho$ is the volumetric water content 14 (m³ m⁻³), and *w* the gravimetric water content (kg kg⁻¹). For soils where compaction was 15 suspected the bulk density was corrected (ρ_c) using the average air filled porosity for 16 samples where compaction had not occurred (E_c) and calculated using equation (2).

17
$$\rho_c = \frac{1 - E_c}{(w + 1/2.65)}$$
 (2)

For the Glenlea soil E_c was taken as 0.03 and for the Carman site 0.05 when calculating ρ_c . All corrections to bulk density were in 90-120 cm layer of cores identified with compaction.

21

Soil bulk density was lower under the re-established native grassland at Carman in the top
15 cm, below which there were little notable differences between treatments (data not
shown). On the clay soil at Glenlea, soil bulk density was lower under the re-established
native grassland in several layers of the soil profile than the farmed systems (data not

shown). Soil under organic management plots had a tendency towards higher soil bulk
 density in layers deeper than 30 cm, but especially below 60 cm. However, this difference

3 alone was insufficient to affect total profile soil carbon stocks significantly.

4

5 2.5 Soil analysis

6 The remainder of the soil sample was dried at 40° C and then a 40 g subsample finely 7 ground ready for chemical analysis at a commercial soil analysis laboratory (AGVISE, 8 Northwood, North Dakota). Soil was initially analysed for total soil carbon and calcium 9 carbonate content to calculate soil organic carbon by difference (SOC_{diff}) however, the 10 presence of less reactive carbonates produced unreliable estimates of SOC in deeper soil 11 layers. Hence, re-analysis of samples using the Walkley-Black combustion method was 12 used to estimate soil organic matter (SOM_{W-B}) throughout the profile. Excluding samples where carbonate content was more than 0.05% g g^{-1} , regression of total soil carbon against 13 14 SOM_{W-B} was used to convert SOM_{W-B} to SOC. The relationships used were $SOC = SOM_{W-B}$ $_{\rm B}/1.72~({\rm r}^2=0.97)$ at Glenlea and SOC = SOM $_{\rm W-B}/2.60~({\rm r}^2=0.97)$ at Carman. Total 15 16 nitrogen (TN) content was analysed using an elemental vario MAX Carbon-Nitrogen 17 analyser (Elementar, Germany). Soils were also analysed for 0.5 M (pH 8.5) NaHCO₃ 18 extractable plant-available phosphorus (Polsen). Analysis of TN and POlsen was conducted 19 on samples from the upper three sample depth intervals (0-60 cm). Four standard check 20 samples were included in the analysis and levels of variance against standards were acceptable (<0.1% g g⁻¹ for SOC, 0.001% g g⁻¹ for TN, and <7 ppm for P_{Olsen}) 21

22 2.6 Statistical analysis

In each soil layer, stocks of soil carbon, nitrogen and phosphorus were calculated as the product of soil bulk density and the content of each component in that layer. These were then aggregated for the 0-30cm, 0-60 cm and 0-120 cm soil profile to calculate carbon and nutrient stock on an area basis. All statistical analyses were made using Genstat version 12 (VSN International Ltd, Hemel Hempstead, UK). The Carman site was analysed using
ANOVA for a randomized complete block design, while the Glenlea site was analysed
using ANOVA for a completely randomized design. Data from the reestablished native
grassland were omitted to conduct a factorial analysis amongst organic/conventional
management and annual-crop/alfalfa-crop rotations at Glenlea. Multiple comparisons of
treatment means were made using Tukey's test at 95% confidence intervals.

7 **3. Results**

8 3.1 Plant carbon inputs

9 The re-established grassland had the highest NPP of the systems at Glenlea, averaging 6.7 t C ha⁻¹ yr⁻¹ NPP (Table 2). This was 2.8 t C ha⁻¹ yr⁻¹ higher than the next most productive 10 11 system. The higher NPP and low C export from the re-established grassland resulted in much higher C inputs than the other systems (4.4 t C ha⁻¹ yr⁻¹ higher than conventional 12 cropping systems and 5.2 t C ha⁻¹ yr⁻¹ higher than organic cropping systems). This was 13 mainly due to much higher below ground inputs which were 75% (5.4-5.9 t C ha⁻¹ yr⁻¹) of 14 15 total estimated C inputs from the re-established grassland, while inputs from surface 16 residue were similar to annual-crop rotations (Table 2). At both sites and under both management regimes the alfalfa-crop rotations had 0.8-1.3 t C ha⁻¹ yr⁻¹ higher NPP than the 17 18 annual crop rotations, but carbon inputs were similar due to the higher amounts of aboveground biomass removed as hay in the alfalfa crop. Below-ground C inputs under the 19 alfalfa-crop rotation were greater than from surface residue and this was 0.4 t C ha⁻¹ yr⁻¹ 20 more than the corresponding annual-crop rotation. Annual crop rotations had 0.4 t C ha⁻¹ 21 yr⁻¹ higher input of C as surface residue, resulting in similar total C inputs under both 22 23 rotations under the same management regime. At Glenlea, the conventionally managed rotations had 0.8-0.9 t C ha⁻¹ yr⁻¹ higher NPP than the organically managed rotations which 24 corresponded to 0.7 t C ha⁻¹ yr⁻¹ higher C inputs (Table 2). 25

1 INSERT TABLE 2 HERE

2 3.2 Soil profile organic carbon

3 While SOC concentrations were highest in the surface layers (0-30 cm) and declined with depth, the SOC in deeper soil layers were still important contributors to overall soil C 4 stocks. Subsoil layers (30-120 cm) contributed 95-140 t C ha⁻¹ or 50-60% of the SOC stock 5 in the soil profile at Glenlea and 40-50 t C ha⁻¹ or 45% of the SOC stock at Carman (Table 6 3 and 4). At Glenlea surface (0-15 cm) SOC concentrations were 0.3-0.9% g g^{-1} higher in 7 8 some treatments than those measured historically, but sub-surface SOC concentrations 9 were comparable with historical soil surveys at the site (Table 1). SOC concentrations 10 measured at Carman were similar in the surface (0-15 cm), but between 15 and 60 cm they were ~0.2 % g g^{-1} higher than reported in historical soil surveys at the site (Table 1). This 11 12 suggests there may have been a net gain in carbon in these soils over the experimental period. 13

14

15 For the Glenlea experiment, SOC stocks and soil C concentrations to a depth of 1.2 m were 16 the highest under the re-established native perennial grassland, but this was not 17 significantly higher than the conventional annual-crop system (P < 0.05) (Table 3 and 5). The soil profile 0-120 cm under the re-established grassland had an additional 52-65 t C 18 ha⁻¹ compared to organically managed cropping systems, and 40 t C ha⁻¹ more than the 19 20 conventional alfalfa-crop system. Over the life of the study (18 years) this equates to a relative gain of 2.2-3.6 t C ha⁻¹ yr⁻¹ under the re-established grassland compared to these 21 22 systems. While the re-established grassland had the highest soil C concentrations and SOC 23 stocks in the surface layers, this was only significantly higher (P < 0.05) than organic 24 alfalfa-crop rotation in the surface 15 cm (Table 5). Lower soil bulk density in the re-25 established grassland meant that higher SOC concentrations did not translate into higher 26 soil C stocks (data not shown). Differences in soil C stocks and SOC concentrations

1	between systems occurred in deeper soil layers (>30 cm) (Table 5). For example, SOC
2	between 30 and 120 cm the re-established grassland had 18-28 t more C ha ⁻¹ than the
3	conventional systems and 44 t more C ha ^{-1} than the organic systems (Table 3).
4	
5	Comparisons amongst the factorial of organic/conventional management and annual-
6	crop/alfalfa-crop rotations showed that there was both a rotation and management effect
7	but no interaction (Table 3). Organically managed systems had 28 t C ha ⁻¹ lower soil C
8	stocks in the whole profile (0-120cm) and 7 t C ha ⁻¹ less in the surface 30 cm than the
9	conventionally managed systems. Organic systems had a higher proportion of the profile
10	soil C in the surface 30 cm (46%) compared with conventional management (42%). Across
11	both management regimes, the alfalfa-crop rotation had 16 t less soil C ha ⁻¹ to 120 cm and
12	10 t less soil C ha ⁻¹ in the surface 30 cm compared to the annual-crop rotation.
13	
14	Differences in soil SOC stocks between farming systems were smaller at the Carman site
15	than at Glenlea (< 14 t C ha ⁻¹). The annual-crop and alfalfa-crop systems had higher soil C
16	stocks in the top 30 and 60 cm than the re-established native grassland or the fallow (Table
17	4), however there was no significant difference in soil C stocks between treatments below
18	60 cm (20-25 t C ha ⁻¹). The fallow generally had lower SOC concentrations than other
19	treatments but these were only significantly ($P < 0.05$) lower than the alfalfa-crop rotation
20	in the 0-15 cm and 30-60 cm layers, but no other significant differences in SOC
21	concentration were detected (Table 6).
22	
23	INSERT TABLES 3, 4, 5 & 6 HERE
23 24	INSERT TABLES 3, 4, 5 & 6 HERE

At the Glenlea site, there was a positive relationship between total C inputs from the various systems over the experimental period and SOC stocks ($r^2 = 0.745$). This relationship indicated that each t C ha⁻¹ input corresponded to 0.60 t C ha⁻¹ increase in soil C stocks to 120 cm. This rate was much lower if only shallower layers were considered; for each t C ha⁻¹ input SOC stocks increased by 0.36 t C ha⁻¹ in the surface 60 cm ($r^2 =$ 0.82) and 0.15 t C ha in the surface 30 cm ($r^2 = 0.45$). On the other hand, at Carman there was no relationship between C inputs over the experimental period and SOC stocks, indicating a much lower capacity to alter SOC at this site.

7 3.3 Soil profile nitrogen and phosphorus

At both sites there was no significant difference between any farming systems in TN stocks (Table 3 and 4). The highest concentrations of TN were in the surface 15 cm. At Glenlea, there were no significant differences in TN concentrations between production systems in any soil layers (Table 5). At Carman, the alfalfa-crop rotation had significantly higher TN in the surface 15 cm than the fallow, but below this all systems were the same (Table 6).

14 Alfalfa-crop rotations at Glenlea had lower plant available P than annual-crop rotations 15 (organic and conventional) and the re-established native grassland. This was especially 16 evident under organic management, suggesting that removal of biomass as hay without 17 replenishment had depleted soil plant-available P reserves more quickly than grain removal 18 (Table 3). The reduction in P content under the alfalfa-crop rotation occurred in all the top 19 3 soil layers (0-15, 15-30 and 30-60 cm) (Table 5). At Carman, both cropping rotations and 20 the re-established grassland had similar plant available P but the fallow treatment had 13-21 20 kg more available P (Table 4). This was primarily due to higher concentrations in the 22 surface 0-15 cm in the fallow. The annual-crop, alfalfa/crop and re-established native 23 grassland all had similar concentrations of plant available P throughout the soil profile 24 (Table 6).

1 4. Discussion

2 4.1 Soil C stocks were higher under re-established perennial grassland on clay soil 3 The longer term site (Glenlea) had more soil C under land converted from cropping to 4 permanent perennial grass-based pasture compared to land which continued to be cropped. 5 This result is consistent with several other studies in the US and elsewhere around the 6 world (Burke et al., 1995; Dalal et al., 1995; Reeder et al., 1998; Potter et al., 1999; 7 Bowman and Anderson, 2002; Franzluebbers, 2009). This contrasts with one of few other 8 studies of soil C under perennial grass, crested wheatgrass (Agropyron cristatum) on the 9 Canadian prairies which found little or no increase in soil C after 9 years (Curtin et al., 10 2000); though in that study grass was cut for hay which is known to reduce soil C 11 sequestration compared to grazed or unharvested grass pastures (Franzluebbers and 12 Stuedemann, 2009). The estimated difference in soil C balance between the re-established 13 grassland and conventional cropped systems in the present study was between 1.2 and 2.2 t C ha⁻¹ yr⁻¹. This was higher than the 0.5-1.5 t C ha⁻¹ yr⁻¹ reported previously in studies 14 15 investigating C sequestration under grazed or unharvested long-term perennial grass 16 pasture (Potter et al., 1999; Franzluebbers et al., 2000; Gregorich et al., 2001; 17 Franzluebbers and Stuedemann, 2009). However, most of these previous studies measured 18 soil C to shallower depths (e.g. 0-70 cm Gregorich et al. (2001) and 0-20 cm in 19 Franzluebbers et al. (2000)). If only shallower layers are considered similar sequestrations 20 rates were observed here. In the present study 30-40% of the additional C in the soil profile 21 under the re-established grassland was found below 30 cm, which suggests that 22 measurements of only shallow soil layers under perennial grasses may underestimate 23 changes in soil C. 24

25 While higher soil C was found after 18 years of re-established grassland at the Glenlea site,

the grassland had lower soil C stocks than cropped systems after 10 years at the Carman

site. There are several reasons which may explain the different results between the sites. 1 2 Firstly, tillage intensities in the cropping systems differed between the sites, with full 3 tillage each year at Glenlea likely to enhance differences between the grassland and 4 cropping systems compared to the low-disturbance no-till management at Carman (Janzen 5 et al., 1998; McConkey et al., 2003). Few other studies have compared re-established 6 grassland with no-till cropping systems but Franzluebbers et al. (2000) still found after 24 years that a perennial grass pasture had 8 t C ha⁻¹ more soil organic C in the top 20 cm 7 8 compared to a conservation-tillage crop rotation in south-eastern USA. Secondly, the 9 different soil types between the sites (Glenlea on a clay vertisol and Carman on a sandy 10 loam) may have affected soil C sequestration under re-established grassland. Finer-11 textured (clay) soils have greater capacity to gain SOC in the Canadian prairies 12 (McConkey et al., 2003) so the coarse sandy textured soil at the Carman site may have 13 limited the potential to increase soil C. Further, at Glenlea, C gains deeper in soil resulted 14 with re-established grassland suggesting stabilization of root C in the 60 - 120 cm depth. 15 This deeper soil had the lowest concentration of SOC and thus likely to have a greater 16 capacity to increase SOC. Finally, large amounts of surface residues were present in the 17 re-established grassland plots at Carman which has not been accounted for in this study. 18

19 While significant rates of soil C sequestration were measured in this study, this may have 20 been increased further under different management. Firstly, no nutrient inputs were 21 provided to the perennial grassland in this study and hence could have limited plant growth 22 and hence the ability to sequester C. Regular N fertilisation of pasture can increase SOC 23 compared to unfertilised pastures (Reeder *et al.*, 1998), and soil P status has also been 24 attributed to differences in soil C accumulation under perennial grasslands (Bowman and 25 Anderson, 2002). Where significant increases in soil C have been found, legumes were 26 present as a component of the perennial pastures or restored grasslands (Dalal et al., 1995;

Gentile *et al.*, 2005; Franzluebbers and Stuedemann, 2009). Legume content in a perennial
 grass pasture can have a beneficial effect on accumulation of soil C (Robles and Burke,
 1997). In contrast, few legumes were present in the restored grasslands in this study.
 Secondly, grazed pastures increase accumulation of SOC more than in ungrazed pastures
 (Franzluebbers and Stuedemann, 2009). Hence, perhaps the lack of grazing of the re established perennial grassland in this study reduced its capacity to sequester soil C.

7 4.2 Soil C and N stocks were similar in rotations with alfalfa and annual-grain crop only 8 Soil C and nutrient stocks under conventional cropping rotations including alfalfa were no 9 larger than conventional annual crop rotations. Removal of alfalfa hay in this study 10 reduced surface residue inputs of C and N. Instead, grazing alfalfa with livestock could 11 return a greater proportion of C and N and would be beneficial for long-term storage of 12 SOC and TN (Franzluebbers and Stuedemann, 2009). However, other studies elsewhere in 13 the world have also found alfalfa-crop rotations to have little benefit in terms of C 14 sequestration compared to systems based entirely on annual crops (Dalal et al., 1995; 15 Whitbread et al., 2000). This is thought to be because of low residue inputs, especially 16 when heavily grazed or harvested for hay, and their short-term nature with the rapid 17 breakdown and turnover of legume residues and roots. The alfalfa systems in the present 18 study were very short duration involved seeding alfalfa in spring year 1 and terminating in 19 early autumn of year 2 (i.e. 2 growing seasons), which may explain why little benefit to 20 soil C was observed.

21

The alfalfa-crop system also had lower plant-available soil P stocks at the older of the two
experimental sites (Glenlea). This was most severe under organic management, where soil
P concentrations were much lower in the top 60 cm under organic compared to
conventional annual-crop rotations. These results are consistent with previous findings
(Welsh *et al.*, 2009), but this is the first documented case of P depletion also occurring

- 1 below 15 cm in this environment. This observation suggests that P extracting organic
- 2 farming systems may seriously deplete soil P unless remediation actions are taken.
- 3

4 4.3 Soil C and available P were lower under organic management

5 This study found lower soil C under organic compared to conventional management of 6 cropping systems. Other studies that have reported increases in soil C under organic 7 management (e.g. DOK experiment in Switzerland) were attributed to manure additions to 8 soil and greater plant diversity under organic vs conventional management (Fließbach et 9 al., 2007). However, no manure or compost was added to the organic plots sampled in the 10 present study, and hence applied C inputs from external sources were no greater than in the 11 conventional system. A more comparable organic legume-based cropping system was 12 implemented at the Rodale Institute Farming Systems Trial, which did not add manure but 13 relied on nitrogen-fixing green manure crops as an N source (Pimentel et al., 2005). After 14 22 years, they found the legume-based organic system had 15% higher soil C in the top 30 cm of soil, accumulating 280 kg ha⁻¹ more organic carbon than the conventional cropping 15 16 system, whilst plant C inputs were comparable between both systems (Pimentel et al., 17 2005). In contrast, residue inputs from the organic systems were 60-65% of conventional 18 cropping systems in this study (Table 3). We are unaware of any other studies that have 19 compared subsoil C between organic and conventionally managed agricultural systems. 20

21 **5. Conclusions**

This paper is one of the first to look at the effects of agricultural management on surface and sub-soil organic carbon on the Canadian Prairies. Re-establishment of grassland on a clay, but not sandy loam, soil resulted in more SOC than the cropped systems in southern Manitoba. This was attributable to higher estimated above and below ground inputs from the perennial grassland. SOC in the clay soil occurred deep in the soil profile (30-120 cm)

1 indicating a higher capacity of the soil to sequester C in the presence of deep plant roots. 2 Hence, studies examining only surface layers (0-30 cm) are likely to underestimate the C 3 sequestration under perennial grasslands. Using short phases of the perennial forage 4 legume, alfalfa (1.5 years), in a cropping sequence failed to increase soil C or total soil N 5 and depleted plant available P compared with rotations involving only annual grain crops. 6 Removal of alfalfa biomass as hay reduced surface C and N inputs and exported P. 7 Without manure and compost additions organically managed cropping systems had lower 8 stocks of SOC than conventionally managed systems. The lower stocks of SOC were 9 associated with reduced estimated C input from crop residue and roots. These findings 10 highlight that organic management can reduce SOC compared to conventional cropping 11 systems unless C inputs are maintained which may require manure or compost additions. 12 In particular, animal manures and compost not only provide a source of C but replenish 13 plant available P concentrations to sustain crop productivity.

14

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24

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4

1 **Tables and Figures**

2 **Table 1.** Soil textural and chemical characteristics from historical soil surveys conducted

3 nearby experimental locations at Carman and Glenlea Research Stations (Michaylna, 1963;

Soil depth (cm)	Sand %	Silt %	Clay %	pH (1:5 CaCl ₂)	EC (ms cm ⁻¹)	OC ^a (%)	Total N (%)	CEC	CaCO ₃ (%)	
Glenlea Sca	Glenlea Scanterbury soil series									
0-18	6	23	71	6.5	-	2.59	0.31	52	0.3	
18-55	9	16	75	7.2	0.41	1.48	0.15	50	0.2	
55-70	7	17	76	7.7	0.47	0.49	0.09	45	2.6	
70-120	2	14	84	8.1	0.53	0.6	0.06	41	11.7	
Carman Hoo	chfeld so	il series								
0-16	81	5	14	5.5	n/a	1.55	n/a	n/a	0	
16-30	87	5	8	-	-	0.85	-	-	0	
30-60	91	4	5	-	-	0.27	-	-	0.5	
60-90	83	11	6	7 0	-	-	-	-	8.7	
90-120	60	34	6	1.0					21.4	

4 Mills and Haluschak, 1993).

5 n/a – historical data not available; ^a – Walkley-Black method

1	Table 2. Cumulative amounts of C fixed (t C ha ⁻¹) as net primary production (NPP), in
2	plant residue returned to soil surface (C_S), and below ground as root tissue (C_R) or extra-
3	root material (C_E), and total C input (C_i) under different systems at Glenlea over 18 years
4	and Carman over 9 years. Methods outlined by Bolinder et al., (2007) were used to
5	estimate NPP and plant C allocations; for further detail refer to section 2.3.

Site - System	Total NPP	C _S ^a	$C_R + C_E$	C_i
Glenlea				
Conventional annual-crop	55.0	23.8	16.8	40.6
Conventional alfalfa-crop	72.1	17.1	23.4	40.5
Organic annual-crop	30.9	18.6	8.6	27.2
Organic alfalfa-crop	46.2	11.8	15.2	27.0
Re-established grassland ^b	119.7	23.0	91.8	114.8
Carman				
Annual-crop	33.1	14.9	9.3	24.2
Alfalfa-crop	45.0	8.5	18.1	26.6
Re-established grassland ^c	-	-	-	-
Fallow	0	0	0	0

^a includes 15% loss of harvested grain or forage and other above-ground C; ^b assumes surface residues were removed in years where grassland was burnt; ^c above-ground biomass was not measured.

8 9

Table 3. Stocks of organic carbon, total nitrogen, and extractable P (Olsen) at Glenlea after 18 years under alfalfa/crop and annual-crop rotations
 managed organically and conventionally, and re-established native grassland. Letters denote significant differences between treatments within each
 depth increment (*P*<0.05); where no letters are provided there was no significant difference between treatments. Analysis of variance (ANOVA) for the

4 main factors of the factorial analysis of management (organic vs conventional) and rotation (alfalfa/crop vs annual crop) and their interaction are

Soil	Organic		Conver	Conventional		AN	ANOVA (P value)		
depth (cm)	Alfalfa/crop	Annual crop	Alfalfa/crop	Annual crop	- grassland	Management	Rotation	Man. \times Rot.	
Organic (Organic C stocks (t ha ⁻¹)								
0-30	$71~\pm 5^{~b}$	84 ± 3 ^{ab}	$80\pm4^{\ ab}$	$89\pm3~^a$	92 ± 5 ^a	**	***	n.s	
0-60	$119\pm7^{\ b}$	127 ± 6^{b}	127 ± 6^{b}	141 ± 5^{ab}	$156\pm8^{\ a}$	*	*	n.s	
0-120	165 ± 11 ^c	$178\pm 6^{\ bc}$	$190\pm7~^{bc}$	$209\pm7~^{ab}$	$230\pm10^{\ a}$	***	*	n.s.	
Total N st	$tocks (t ha^{-1})$								
0-30	7.2 ± 0.3	6.8 ± 0.3	6.4 ± 0.5	7.7 ± 0.3	6.9 ± 0.3	n.s	n.s	**	
0-60	8.4 ± 0.8	6.9 ± 0.3	7.9 ± 0.8	8.4 ± 0.8	8.6 ± 0.8	n.s	n.s	n.s	
Total plant available $P(kg ha^{-1})$									
0-30	$9.2\pm0.7~^{c}$	$47.0\pm4.8\ ^{a}$	$28.0\pm3.0\ ^{b}$	$43.6\pm4.0~^a$	$42.4\pm3.9~^a$	**	***	***	
0-60	13.5 ± 0.8^{c}	53.0 ± 5.2^{a}	$32.6\pm3.1^{\ b}$	51.4 ± 4.8^{a}	$48.2\pm4.1~^a$	**	***	**	

5 presented (factorial analysis excludes re-established grassland treatment).

n.s. - not significant, *** - P<0.01, ** - P<0.05, * - P<0.1

1 **Table 4.** Stocks of organic carbon, total nitrogen, and extractable P (Olsen) at Carman

- 2 after 9 years under alfalfa/crop rotation, annual-crop rotation, re-established native
- 3 grassland, and chemical fallow. Letters denote significant differences between treatments
- 4 within each depth increment (P < 0.05); where no letters are provided there was no
- 5 significant difference.

6

Soil depth (cm)	Alfalfa/crop	Annual crop	Native grassland	Fallow					
Total or	Total organic C stocks (t ha ⁻¹)								
0-30	54 ± 1^{a}	54 ± 1 ^a	$48\pm2~^{b}$	47 ± 2^{b}					
0-60	77 ± 2 ^a	77 ± 2 ^a	69 ± 2 ^b	68 ± 2^{b}					
0-120	99 ± 4^{a}	102 ± 3 ^{ab}	90 ± 3 bc	88 ± 4 ^c					
Total N	$stocks (t ha^{-1})$								
0-30	4.6 ± 0.2	4.1 ± 0.3	3.9 ± 0.2	3.7 ± 0.3					
0-60	6.7 ± 0.4	6.0 ± 0.5	5.9 ± 0.3	$5.7\ \pm 0.5$					
Total plant available $P(kg ha^{-1})$									
0-30	$23.2\pm2.2~^{b}$	$20.3\pm1.9~^{b}$	$22.2\pm1.7~^{\rm b}$	$37.4\pm3.1~^{a}$					
0-60	$31.2\pm2.9~^{b}$	$25.6\pm2.4~^{b}$	$29.8\pm2.4~^{b}$	$44.4\pm3.6~^a$					

Table 5. Soil organic carbon, total nitrogen, and extractable P (Olsen) concentrations
 throughout the soil profile at Glenlea after 18 years under alfalfa/crop and annual-crop
 rotations managed organically and conventionally, and re-established native grassland.
 Letters denote significant differences between treatments within each depth increment

(P < 0.05); where no letters are provided there was no significant difference.

Soil depth	Organic		Conver	Re-established grassland	
(cm)	Alfalfa/crop	Annual crop	Alfalfa/crop	Annual crop	-
Organic C	concentration (g	kg^{-1})			
0-15	24.3 ± 1.4 ^b	$29.7\pm0.6~^a$	$29.5\pm1.4~^{a}$	$32.9\pm0.5~^a$	$34.4\pm2.1~^a$
15-30	16.3 ± 2.0	18.7 ± 1.6	17.1 ± 1.7	19.2 ± 1.6	20.9 ± 1.6
30-60	$11.7\pm1.2~^{\rm b}$	$10.7\pm0.9~^{\rm b}$	$12.1\pm0.8~^{ab}$	$13.0\pm0.7~^{ab}$	$16.8\pm1.2~^a$
60-90	$6.7\pm0.8~^{c}$	$6.9\pm0.7~^{bc}$	$8.6\pm0.6~^{abc}$	$9.3\pm0.5~^{ab}$	$10.9\pm0.7^{\ a}$
90-120	$3.8\pm0.7~^{b}$	$4.5\pm0.4~^{b}$	$6.6\pm0.3~^a$	$6.6\pm0.2~^a$	$7.2\pm0.3~^a$
Total N con	ncentration (g kg	-1)			
0-15	2.65 ± 0.11	2.59 ± 0.05	2.67 ± 0.09	2.74 ± 0.07	2.58 ± 0.09
15-30	1.51 ± 0.20	1.34 ± 0.13	1.13 ± 0.22	1.72 ± 0.16	1.58 ± 0.18
30-60	0.31 ± 0.14	0.08 ± 0.08	0.40 ± 0.15	0.19 ± 0.13	0.45 ± 0.17
Plant avail	able P concentra	tion (mg kg ⁻¹)			
0-15	$3.60\pm0.41~^{\rm c}$	$23.4\pm2.1~^{a}$	14.2 ± 1.57 $^{\rm b}$	$21.7\pm1.9\ ^{a}$	$20.9\pm2.3~^{ab}$
15-30	$1.73\pm0.23~^{c}$	$4.53\pm0.92~^a$	$2.80\pm0.38~^{ab}$	$4.20\pm0.66\ ^a$	$5.13\pm0.65~^a$
30-60	$1.07\pm0.07~^{b}$	$1.53\pm0.22~^{ab}$	$1.20\pm0.11~^{b}$	$2.00\pm0.31~^a$	$1.53\pm0.17~^{ab}$

 6^{-a} - concentrations were very low below 60 cm and are not presented

Table 6. Soil organic carbon, total nitrogen, and extractable P (Olsen) concentrations
 throughout the soil profile at Carman after 9 years under alfalfa/crop rotation, annual-crop
 rotation, re-established native grassland, and chemical fallow. Letters denote significant
 differences between treatments within each depth increment (*P*<0.05)); where no letters

Soil depth (cm)	Alfalfa/crop	Annual crop	Re-established grassland	Fallow					
Organic C c	Organic C concentration $(g kg^{-1})$								
0-15	$14.8\pm0.4~^a$	14.3 ± 1.1 ^a	$14.1\pm0.4~^{ab}$	$11.9\pm0.9~^{b}$					
15-30	11.7 ± 0.4	10.6 ± 0.8	10.8 ± 0.5	9.7 ± 0.8					
30-60	$5.9\pm0.3~^a$	$4.9\pm0.4~^{ab}$	$5.0\pm0.2~^{ab}$	$4.5\pm0.4~^{b}$					
60-90	2.9 ± 0.3	3.2 ± 0.3	2.6 ± 0.2	2.4 ± 0.3					
90-120	2.1 ± 0.2	2.2 ± 0.2	2.0 ± 0.3	1.8 ± 0.2					
Total soil N	concentration (g	kg^{-1})							
0-15	$1.27\pm0.06~^a$	$1.21\pm0.06~^{ab}$	$1.11\pm0.05~^{ab}$	$1.03\pm0.04~^{b}$					
15-30	0.97 ± 0.05	0.93 ± 0.03	0.88 ± 0.05	0.88 ± 0.06					
30-60	0.52 ± 0.07	0.46 ± 0.06	0.48 ± 0.04	0.52 ± 0.03					
Plant available P concentration (mg kg^{-1})									
0-15	$8.80\pm0.85^{\ b}$	$8.36\pm0.58^{\ b}$	9.20 ± 0.78^{b}	15.86 ± 0.66^{a}					
15-30	2.67 ± 0.27	2.43 ± 0.28	2.53 ± 0.29	3.43 ± 0.24					
30-60	1.93 ± 0.21	1.29 ± 0.12	1.80 ± 0.31	1.79 ± 0.18					

5 are provided there was no significant difference.

Annual-crop

Alfalfa/crop



Figure 1. Crop sequences used for annual-crop and alfalfa/crop rotations at (a) Glenlea and
(b) Carman long-term farming systems experiments in southern Manitoba. Dotted arrow
indicates organic management where a fababean green manure replaced a soybean grain
crop in the annual-crop rotation at Glenlea.