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Carbon dioxide flush as a soil health indicator related to soil properties and crop yields

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Abstract

Carbon dioxide flush after rewetting of dried soils has been recommended as a promising soil health indicator, but it has not been related to most soil properties and crop yields. We evaluated the effect of cropping systems and N fertilization on CO_2 flushes at 1- (1dC) and 4-d incubations (4dC) after rewetting of dried soils and related to 54 soil physical, chemical, and biological properties and annualized crop yields in two long-term experimental sites in eastern Montana (USA). Treatments included till and no-till spring wheat (*Triticum aestiveum* L.), pea (*Pisum sativum* L.), and fallow rotations with and without N fertilization. Carbon dioxide flushes were lower in till crop–fallow than in no-till continuous cropping systems at both sites. The 1dC was correlated to 5 soil physical, 7 chemical, and 12 biological properties, and 4dC was correlated to 9 physical, 8 chemical, and 11 biological properties in Froid. In Sidney, 1dC was correlated to 10 physical, 13 chemical, and 9 biological properties, and 4dC was correlated to 7 physical, 11 chemical, and 2 biological properties (1–8 moderately, 18–21 strongly, and 1–3 very strongly related). Carbon dioxide flushes were also related to mean annualized crop yields in both sites, except for the relationship between 4dC and crop yield in Sidney. Because of its stronger relationship with soil properties and crop yields, 1dC after rewetting of dried soils determined by using the infrared gas analyzer can be used as a simple, rapid, reliable, and inexpensive indicator of measuring soil health in dryland cropping systems.

1 | INTRODUCTION

Carbon dioxide flush after rewetting of dried soil, which measures microbial activity, has been proposed as one of the most promising soil health indicators (Franzluebbers, 2016; Moebius-Clune et al., 2017). Although soil organic C takes a long time to change with management practices and climatic conditions due to large pool size and inherent spatial variability, labile fractions, which can be indexed via CO_2 evolution, change rapidly within a growing season and are sensitive measures of changes in soil organic matter (Franzluebbers, 2016; Franzluebbers et al., 2000). The flush of CO_2 after rewetting of dried soil may indicate nutrient cycling, C sequestration, decomposition of organic matter, natural and organic amendments, amount and quality of substrate availability, size of

Abbreviations: 1dC, CO_2 flush at 1-d incubation determined by using the infrared analyzer; 4dC, CO_2 flush at 4-d incubation determined by the alkali-trap method; ACEP, autoclaved citrate-extractable protein; CASH, Cornell Comprehensive Assessment of Soil Health; CTWF, conventional till barley/spring wheat–fallow; FSTCW, fall and spring till continuous spring wheat; NABG, N-acetyl β-glucosaminidase; NTCW, no-till continuous barley/spring wheat; NTWF, no-till barley/spring wheat-fallow; NTWP, no-till barley–pea (1984–1999) replaced by spring wheat–pea (2000–2019); STWF, spring till spring wheat–fallow

microbial biomass pool, N mineralization potential, and soil aggregation (Franzluebbers et al., 2000).

Soil quality or soil health assessment frameworks mostly include a suite of soil physical and chemical properties (Doran & Parkin, 1994; Karlen et al., 1997) without much emphasis on biological properties (Franzluebbers, 2016). These assessments include the Soil Health Management Assessment Framework (Andrews et al., 2004), Haney Soil Test (Haney et al., 2010), and Cornell University's Comprehensive Assessment of Soil Health (CASH) (Moebius-Clune et al., 2017). These assessments often do not consistently measure soil health in all regions due to variations in soil and climatic conditions and management practices (Doran & Parkin, 1994; Karlen et al., 1997). The CASH measurement includes unweighted-average approach of individual soil attributes, which could provide biased health score due to extreme values of some properties (Congreves et al., 2015; Idowu et al., 2008). Soil tests used to measure soil fertility include mostly chemical properties and application of chemicals, such as fertilizers, that can degrade physical and biological properties (Karlen et al., 1997; Tilman et al., 2002). A healthy soil should enhance crop yields during favorable weather condition and withstand against nutrient and yield losses and degradation of environmental quality during extreme weather (Congreves et al., 2015). Few indicators of measuring soil health that are linked to biogeochemical functions and crop yields will be more meaningful to producers compared with a suite of physical, chemical, and biological properties that are time consuming, expensive to measure, and unlinked to biogeochemical functions or crop yields (Franzluebbbers et al., 2000). The soil health indicator should be (a) easy to measure, (b) sensitive to management practices and soil and climatic conditions, (c) inexpensive, (d) easily accessible to producers, and (e) relate to soil properties and crop yields (Doran & Parkin, 1994 ; Franzluebbers, 2016).

The CO_2 flush at 1-d incubation (1dC) after rewetting of dried soil is one of such soil health indicators that is easy to measure, simple, inexpensive, reliable, and relates to soil N mineralization and availability, microbial biomass, and soil organic matter (Castellanos & Pratt, 1981; Haney et al., 2001; Franzluebbers et al., 2000). Because it takes a long time to measure N mineralization potential and farmers often do not take account for soil N mineralization while applying N fertilizers to crops, excessive N fertilization can often lead to increased accumulation of soil residual N that can degrade soil and environmental quality. Measurement of CO_2 flush can estimate N mineralization potential of the soil that can be used to adjust N fertilization rates to crops, reducing the degradation of soil and environmental quality (Franzluebbers et al., 2000; Haney et al., 2001; Mac Bean et al., 2020). The CO_2 flush is also affected by soil bulk density (Franzluebbers, 1999; Torbert & Wood., 1992) and water content, as these influence microbial activity (Franzluebbers, 1999; Harris, 1981; van Es & Karlen, 2019). Liebig et al. (1995) reported that soil respiration measured by using gas chromatograph was negatively correlated to bulk density, but positively to saturated hydraulic conductivity, soil aggregation with <1.0-mm aggregates, sand concentration, and water content at <47% water-filled pore space. At 61–73% water-filled pore space, they found that soil respiration was negatively correlated to soil organic C and total N. The CO_2 flush was also correlated to extractable P (Alves de Castro Lopes et al., 2013), soil organic matter (Yost et al., 2018), aggregate stability, autoclaved-extractable protein (ACEP), $KMnO_4$–extractable C, pH, P, K, Mg, and Mn concentrations (van Es & Karlen, 2019), water-soluble C, K_2SO_4–extractable C, and microbial biomass C (Wang et al., 2003).

Soil CO_2 flush had a variable relationship with crop yield and N uptake. The 1dC was related to forage N uptake (Haney et al., 2001), CO_2 flush at 3-d incubation to dry matter production (Franzluebbers, 2016), and the flush at 4-d incubation (4dC) to corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] yields (van Es & Karlen, 2019). Similarly, Alves de Castro Lopes et al. (2013) reported that CO_2 flush at 7-d incubation was related to crop yield in Brazil. Several researchers (Mac Bean et al., 2020; Yost et al., 2018) found that 4dC was moderately related to economical optimum N rate for corn production. In contrast, Roper et al. (2017) found that 4dC was not related to crop yields in North Carolina. They noted that lower crop yields due to non-soil factors, such as variations in air temperature and precipitation and crop damage due to pest infections, make the relationship between CO_2 flush and crop yield challenging. Therefore, long-term data are needed to evaluate the relationship where mean yield across years can be used so that non-soil factors will have little effect on the

Core Ideas

- A rapid and inexpensive soil health indicator that relates to soil properties and crop yield is needed.
- Relationships between CO_2 flushes, soil properties, and crop yields were examined in long-term experiments.
- CO_2 flushes were related to 11 soil physical, 26 chemical, and 11 biological properties and mean crop yield.
- CO_2 flush at 1-d incubation was related to five more soil properties and two more crop yields than at 4-d incubation.
- CO_2 flush at 1-d incubation can be used as a rapid and inexpensive indicator of soil health in dryland farming.

relationship (Alves de Castro Lopes et al., 2013; Congreves et al., 2015; van Es & Karlen, 2019).

The relationships between soil CO_2 flush after rewetting of dried soils, soil physical, chemical, and biological properties, and crop yields are still lacking, especially in long-term experiments under dryland cropping systems in arid and semiarid regions. This study evaluated the relationships between 1dC determined by the infrared analyzer and 4dC determined by the alkali trap method after rewetting of dried soils, 54 soil physical, chemical, and biological properties, and mean annualized crop yields in two long-term (14- to 36-yr-old) experiments under dryland cropping systems in eastern Montana. Our objectives were (a) to examine how tillage, cropping system, and N fertilization affected 1dC and 4dC, (b) to determine if 1dC and 4dC are related to soil physical, chemical, and biological properties and mean annualized crop yields across years, and (c) to evaluate if 1dC determined by the infrared analyzer is better than 4dC determined by the alkali trap method for relating to soil properties and crop yields. We hypothesized that 1dC would be more sensitive to treatments and better related to soil properties and crop yields than 4dC.

2 | MATERIALS AND METHODS

2.1 | Experimental sites, treatments, and management

The two long-term experiments were established in dryland farming sites in eastern Montana (USA). The 36-yr-old site in Froid, MT (48°20′ N, 104° 29′ W) was established in 1983 (Aase & Pikul, 1995), where the soil was a Dooley sandy loam (fine loamy, mixed, frigid, Typic Argiborolls) with 645 g kg^{-1} sand, 185 g kg^{-1} silt, 170 g kg^{-1} clay, 14.9 g kg^{-1} soil organic C, and 6.2 pH at the 0-to-15-cm depth. The mean monthly air temperature at the site is 8 ˚C and annual precipitation is 357 mm. The 14-yr-old site in Sidney, MT (48°33′ N, 104°50′ W) was established in 2006 (Sainju & Alasinrin, 2020), where the soil was a Williams loam (fine-loamy, mixed, superactive, frigid, Typic Argiustolls), with sand, silt, and clay concentrations of 350, 325, and 325 g kg^{-1} respectively, pH of 7.2, and soil organic C of 13.2 g kg^{-1} at the 0-to-20-cm depth. Mean monthly air temperature at the site is also 8 ˚C, with an annual precipitation of 340 mm. The two sites are about 88 km apart.

Treatments in Froid, MT, included a combination of tillage and cropping systems and were fall and spring till continuous spring wheat (FSTCW), no-till continuous spring wheat (NTCW), no-till spring wheat-barley (*Hordeum vulgaris* L.) (1984–1999) replaced by spring wheat–pea (2000–2019) (NTWP), and spring till spring wheat–fallow (STWF), with each crop phase occurring in each year. Fall tillage included tilling plots with a tandem disc and spring tillage with a field cultivator to a depth of 8 cm. Although herbicides were used to control weeds in all treatments, additional tillage occurred during the fallow period to control weeds in STWF, the conventional cropping system in the region. The experiment was a randomized block design with four replications of treatments and a plot size of 12 × 30 m. Detailed description of crop management is shown in Aase and Pikul (1995). In brief, spring wheat, barley, and pea were planted at recommended seed rates in the last week of April and harvested in late July to mid- August in each year. Seeds were planted at 20-cm spacing using a double disk opener from 1984 to 1996 and a John Deree no-till drill from 1997 to 2019. Nitrogen fertilizer was broadcast to spring wheat and barley at planting from 34 to 70 kg N ha^{-1} depending on soil residual N content (to a depth of 60 cm determined in the autumn of the previous year) to achieve available N content of 80 kg N ha^{-1} for barley and 100 kg N ha^{-1} for spring wheat (recommended N rates) from the soil and fertilizer. No N fertilizer was applied to pea. Phosphorus and K fertilizers were banded at 11 kg P ha^{-1} and 29 kg K $ha^{-1,}$ respectively, 5 cm to the side and 5 cm below seeds to all crops at planting every year. Crop yields were determined by harvesting bundled samples (1984–1995) or with a combine (1986–2019) from an area of 1.5 m × 20.0 m. Yields were adjusted to oven-dried basis (65 ˚C for 7 d), and crop residue was returned to the soil after grain harvest.

In Sidney, MT, main-plot treatments were conventional till (or spring till) barley/spring wheat–fallow (CTWF, traditional system), no-till barley/spring wheat-fallow (NTWF), no-till continuous barley/spring wheat (NTCW), and no-till barley/spring wheat–pea (NTWP) and split-plot treatments were 0 and 80/100 kg N ha^{-1}. Barley was grown from 2006 to 2011, which was replaced by spring wheat from 2012 to 2019 in all cropping systems. All phases of crops in the rotations were present in each year. Plots in CTWF were tilled with a field cultivator to a depth of 8 cm to prepare seedbeds and during the fallow period to control weeds. Nitrogen fertilizer was applied to barley at 0 and 80 kg N ha^{-1} from 2006 to 2011 and to spring wheat at 0 and 100 kg N ha^{-1} from 2012 to 2019. As in Froid, MT, N fertilization rates were adjusted to soil residual N to a depth of 60 cm determined in the autumn of the previous year. Pea did not receive N fertilizer. The experiment was split-plot design in a randomized block with three replications of treatments. The split plot size was 12.0 × 6.0 m. All crops were planted in late April in each year at recommended seed rates with a no-till drill at a row spacing of 20 cm. At planting, P and K fertilizers were banded at 11 kg P ha^{-1} and 27 kg K ha^{-1}, respectively, 5 cm to the side and 5 cm below seeds to all crops. Herbicides and pesticides were applied as needed. Crops were harvested in late July to mid-August every year with a combine from a swath of 11.0 × 1.5 m and grain yields were determined on an

oven-dried basis (65 ˚C for 7 d). After grain harvest, crop residues were returned to the soil. Further details are shown in Sainju and Alasinrin (2020).

2.2 | Soil sampling and analysis

At both sites, soil samples were collected in April 2019 before tillage, planting, and fertilization. A sharpshooter spade (38-cm × 15-cm blade) was used to dig six holes (15 × 15 cm) to a depth of 15 cm in a zigzag pattern covering row and inter-row within a plot. Using a soil knife, a slice of soil (4-cm wide × 1.5-cm thick × 15-cm deep) from three sides of the hole was removed, placed in a plastic bag, and carried in a cooler with ice. Ninety-six soil samples were collected in Froid and 144 samples were collected in Sidney. The bulk soil within a plot was mixed in a container sterilized with isopropyl alcohol and passed through a 8-mm sieve. About 400 g of soil in a plastic bag from each plot was put in a cooler packed with ice and shipped to soil testing laboratories for analysis of biological properties. The remaining soil was shipped to other laboratories where soils were air dried, ground, and sieved to 2 mm before analyzing physical and chemical properties.

Soil bulk density and water holding capacity were determined by driving four cores (7.6-cm diam., 7.6-cm deep) with a hammer covering row and inter-row near four holes in each plot. Two cores were oven dried at 105 ˚C for 24 h, and the bulk density was determined by dividing the weight of oven-dried soil by the volume of the core (Blake & Hartge, 1986). The other two cores were used to determine volumetric water content at the field-moist condition, water saturation, and at 0.3, 10, 33, and 1,500 kPa pressure using the pressure-plate technique (Reynolds & Topp, 2008). Measurements were made for intact and repacked soil cores. Water holding capacity for repacked and intact soil cores was determined as the difference between water contents at 10 and 1,500 kPa (Cassel & Neilson, 1986). Soil bulk density and volumetric water contents for a treatment were determined by averaging the values from two cores. Saturated hydraulic conductivity was determined in the field using the two-ponding head method with a device (SATURO, Meter Group) by measuring the water flow rate to a depth of 10 cm after saturation (Reynolds & Elrick, 1990).

Sand, silt, and clay concentrations were determined by the pipette method (Gee & Bauder, 1986). Aggregate stability was determined as the amount of 0.25-to-2.00-mm aggregates remaining in a 0.25-mm sieve after a 5 min simulated hard rainfall (Schindelbeck et al., 2016). Water-stable aggregate was determined as the amount of 1-to-2-mm aggregates remaining in a 0.25-mm sieve after oscillation in water for 3 min (Kemper & Rosenau, 1986). Dry and wet stability of aggregates were determined by dividing the mean-weight diameter of dry- and wet-sieved aggregates by the total weight of aggregates, respectively (Franzluebbers et al., 2000). Average slake aggregate was determined using the smartphone app "SLAKES" after spreading soil aggregates in a plate and coinciding the picture of aggregates with the application barcode that measures aggregation (Fajardo et al., 2016). The amount of stone in the soil based on volume using the water displacement technique and based on weight were determined as described by Hao et al. (2008). Macro- and mesoporosity were determined as pore space >1 mm and 10 μm, respectively (Topp et al., 1993). Total shrinkage was determined by adding beads in the soil and volume lost after oven drying the sample at 110 ˚C for 24 h (Hao et al., 2008).

Soil pH and electrical conductivity were determined using a pH meter (1:2 soil/water ratio) (Thomas, 1996). Soil Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, S, and Zn concentrations were determined by extracting the soil with Mehlich 3 solution and quantification by inductive coupled plasma–atomic emission spectroscopy (ICP–AES) (Sikora & Moore, 2014). Soil inorganic and organic P concentrations were measured using the H3A extract, followed by quantification with ICP–AES (Haney et al., 2010). Sodium absorption ratio was determined using the saturated paste extract, followed by quantification of Na, Ca, and Mg by ICP–AES (Miller et al., 2013). Soil total C and N concentrations were determined using dry combustion in a C and N analyzer (Nelson & Sommers, 1996). Soil organic C concentration was determined on samples treated with 6 mol L^{-1} HCl and dry combustion as above. Soil organic matter was calculated by multiplying soil organic C by a factor of 1.724. Water-extractable organic N and total C and N were determined as shown by Haney et al. (2012). The NH_4–N and NO_3–N concentrations were determined by using the H3A extract (Haney et al., 2012) and measuring in an autoanalyzer. The $KMnO_4$–extractable C was determined by oxidizing the soil in $KMnO_4$ solution, followed by color absorption in a colorimeter (Weil et al., 2003).

The 1dC was determined by measuring the CO_2 flush using the infrared gas analyzer (Haney et al., 2008) and 4dC by measuring the flush using an alkali (KOH) trap (Zibilske, 1994) from rewetted dry soils at 50% water-filled pore. For the infrared gas analyzer, 40 g soil sample was wetted with water to 50% water-filled pore space in a 250-ml jar and closed with lids with ports where two solenoids were connected to infrared gas analyzer (Haney et al., 2008). The CO_2 flush was measured by the analyzer every hour for 24 h at a rate of 400 ml min^{-1} for 3 min. For the alkali-trap method, 40 g soil was wetted with water to 50% water-filled pore space in a 100-ml beaker, placed in a 1-L mason jar containing 10 ml of 1 M KOH and 10 ml of deionized water to maintain humidity (Zibilske, 1994). The soil was incubated for 4 d and CO_2 absorbed by KOH was back-titrated with 1 M HCl using phenolphthalein as the indicator. Potentially mineralizable N was determined by measuring NH_4–N

concentration in 7-d anaerobic soil incubation at 40 ˚C (Bundy & Meisinger, 1994). The autoclaved citrate-extractable protein (ACEP) was measured as citrate-extractable and autoclaved protein at 121 ˚C for 30 min, followed by using spectracount colorimetric microplate reader using bovine serum albumin as the standard (Schindelbeck et al., 2016). The potential activity of β-glucosidase (Tabatabai, 1994), N-acetyl β-glucosaminidase (NABG) (Deng & Popova, 2011), phosphomonoesterase (Acosta-Martinez & Tabatabai, 2011), and arylsulfatase (Klose et al., 2011) enzymes were determined in soils incubated with standard solution of p-nitrophenol at 37 ˚C for 1 h, followed by colorimetric determination of the color.

2.3 | Statistical analysis of data

Data for 1dC and 4dC were analyzed using a MIXED procedure of SAS after checking for normal distribution of residuals (Littell et al., 2006). For Froid, cropping system was considered as the fixed effect and replication as the random effect for data analysis. For Sidney, cropping system was considered as the main-plot and N fertilization as the split-plot treatment. Fixed effects were cropping system, N fertilization, and cropping system × N fertilization interaction and random effects were replication and replication × cropping system interaction. The least square means test (Littell et al., 2006) was used to separate means and interactions when significant. Pearson's correlation analysis was used to correlate 1dC and 4dC with soil physical, chemical, biological, and biochemical properties. For most significant soil properties that correlated with CO_2 flushes at both sites, regression analysis was conducted among 1dC, 4dC, and soil properties for data combined from both sites. For this, soil properties were considered as independent variables and 1dC and 4dC as the dependent variables. Regression analysis was also conducted among 1dC, 4dC, and mean annualized crop yields across years for data from individual and combined sites to find their relationships. As crop yields were tested as response variables against CO_2 flushes, 1dC and 4dC were considered as independent variables and crop yields as dependent variables for the regression analysis. Annualized crop yield for a treatment was calculated by averaging yields of all crops within a rotation in a year. For this, crop yield during the fallow phase was considered zero due to the absence of crops. For example, annualized crop yield in CTWF in a year is the average of spring wheat yield during the crop phase and fallow phase (or spring wheat yield/2, as yield during the fallow phase is zero). Data were considered statistically significant at $P \leq .05$, unless mentioned otherwise. Threshold P values were adjusted for multiple comparisons. Comparison between sites was made based on treatment response to 1dC and 4dC and number of relationships among 1dC and 4dC, soil properties, and crop yields.

TABLE 1 Effect of 36 yr of tillage and cropping system combination on soil CO_2 flush at 1- and 4-d incubations (1dC and 4dC, respectively) (mean ± standard deviation) after rewetting of dried soil (sandy loam) in Froid, MT

Tillage and cropping system[a]	1dC	4dC
	mg CO_2–C kg^{-1}	
FSTCW	47.8 (± 11.0)a[b]	440.5 (± 93.6)a
NTCW	49.1 (± 5.6)a	370.2 (± 37.3)ab
NTWP	57.9 (± 24.7)a	371.5 (± 108.0)ab
STWF	26.7 (± 5.3)b	260.2 (± 60.2)b
P value	.049	.026

[a]Tillage and cropping systems are fall and spring till continuous spring wheat (FSTCW); no-till continuous spring wheat (NTCW), no-till barley–pea (1984–1999) replaced by spring wheat–pea (2000–2019) (NTWP), and spring till spring wheat–fallow (STWF).

[b]Numbers followed by different letters within a column are significantly different at $P \leq .05$ by the least square means test.

3 | RESULTS AND DISCUSSION

3.1 | CO_2 flushes

The 1dC was greater with FSTCW, NTCW, and NTWP than STWF in Froid (Table 1). Similarly, 4dC was greater with FSTCW than STWF. In Sidney, 1dC was greater with NTCW than CTWF and NTWF (Table 2). The 4dC was not affected by tillage and cropping system. Nitrogen fertilization also did not affect 1dC and 4dC in Sidney. The coefficient of variation for 1dC ranged from 11 to 43% in Froid and from 10 to 29% in Sidney. For 4dC, the coefficient of variation ranged from 6 to 30% in Froid and from 8 to 33% in Sidney.

Reduced crop residue input due to the absence of crops during the fallow period likely decreased 1dC and 4dC with STWF in Froid and 1dC with CTWF and NTWF in Sidney. Previous field measurement of CO_2 flux using the static chamber in Sidney also showed that CO_2 flux was lower with crop–fallow than with continuous cropping (Sainju, Caesar-Tonthat, Lenssen, & Barsotti, 2012). Availability of C substrate can affect soil respiration (Wang et al., 2003). Cropping system can influence soil respiration by affecting on the quality and quantity of crop residue returned to the soil (Mosier et al., 2006; Sainju et al., 2010). Nonsignificant difference in 1dC and 4dC between FSTCW and NTCW in Froid and CTWF and NTWF in Sidney suggests that tillage had no effect on CO_2 flush under dryland cropping systems in the semiarid region. This was in contrast with the results obtained by van Es and Karlen (2019), who found that 4dC was greater with conventional tillage than minimum tillage under irrigated cropping systems in the subtropical region in North Carolina. Greater crop residue production and enhanced mineralization of residue due to increased air temperature and precipitation

TABLE 2 Effect of 14 yr of tillage and cropping system combination and N fertilization rate on soil CO_2 flush at 1- and 4-d incubations (1dC and 4dC, respectively) (mean ± standard deviation) after rewetting of dried soil (loam) in Sidney, MT

Variable	1dC	4dC
	mg CO_2–C kg^{-1}	
Tillage and cropping system[a]		
CTWF	26.8 (± 6.1)b[b]	390.3 (± 63.9)
NTCW	49.6 (± 14.9)a	440.2 (± 35.0)
NTWF	29.7 (± 1.7)b	370.4 (± 121.0)
NTWP	37.0 (± 8.5)ab	400.5 (± 92.3)
N fertilization rate		
0 kg N ha^{-1}	36.0 (± 14.1)	410.2 (± 93.8)
80/100[c] kg N ha^{-1}	35.5 (± 11.4)	390.3 (± 73.5)
	***P* value**	
Cropping sequence (CS)	**.022**	.663
N fertilization rate (NR)	.867	.595
CS × NR	.759	.593

Note. Significant values are shown in bold.

[a]Tillage and cropping systems are conventional till barley/spring wheat–fallow (CTWF), no-till continuous barley/spring wheat (NTCW), no-till barley/spring wheat–fallow (NTWF), and no-till barley/spring wheat–pea (NTWP).

[b]Numbers followed by different letters within a column are significantly different at $P \leq .05$ by the least square means test.

[c]Nitrogen fertilizer was applied at 80 kg N ha^{-1} to barley from 2006 to 2011 and at 100 kg N ha^{-1} to spring wheat from 2012 to 2019.

probably increased CO_2 flush in the subtropical region compared with lower residue production and mineralization of residue due to lower air temperature, limited precipitation, and shorter growing season in our sites. Mean yields of corn and soybean ranged from 2 to 7 Mg ha^{-1} in North Carolina compared with 1.3 to 3.0 Mg ha^{-1} in our sites. As a result, the quantity of residue input was also greater in North Carolina than in our sites. Mean monthly air temperature is 21 °C and annual precipitation is 1,270 mm in North Carolina compared to 8 °C and 350 mm, respectively, in our sites. Our results of nonsignificant difference in 1dC and 4dC between N fertilization treatments in Sidney was also in contrast with that obtained by Sainju, Caesar-Tonthat, Lenssen, and Barsotti (2012) who reported greater flush with 80 than with 0 kg N ha^{-1}. Mac Bean et al. (2020) found that N fertilization decreased 4dC compared with no N fertilization at four sites but did not affect it at 45 sites in U.S. Midwest. Nitrogen fertilization can variably affect CO_2 flux (Al-Kaisi et al., 2008; Sainju et al., 2010).

The 4dC was 6–10 times greater than 1dC. The greater CO_2 flush values with 4dC than 1dC were due to the longer duration of the incubation, as more CO_2 is flushed out and accumulated during the longer incubation period (Haney et al., 2008; Franzluebbers, 2016). The 1dC was also related to 4dC in Froid ($R^2 = .42$, $P \leq .001$), but not in Sidney ($R^2 = .002$, $P = .87$), and the combination of data from Froid and Sidney (Figure 1). Differences in the nature of treatments, soil types, length of the experiment between sites, and method of determination of CO_2 flush may have affected the magnitude and correlation between 1dC and 4dC in Froid and Sidney. For example, N fertilization treatment was present in Sidney, but absent in Froid. The length of the experiment was 36 yr in Froid and 14 yr in Sidney. The soil was sandy loam in Froid and loam in Sidney. It may be possible that the nonsignificant effect of N fertilization on CO_2 flux, shorter duration of the experiment, and medium soil texture resulted on nonrelationship between 1dC and 4dC in Sidney compared with Froid where the relationship was significant. Several researchers (Franzluebbers, 2016; Mac Bean et al., 2020) reported that CO_2 flushes at 3- to 4-d incubations were 2.5–3 times greater than at 1-d incubation, and that these flushes were highly related. Although longer duration of the incubation may have increased 4dC compared with 1dC, and soil CO_2 flush measured by the infrared gas analyzer and alkali trap are highly correlated (Haney et al., 2008), the greater 4dC than 1dC in our study compared with those observed by several researchers (Franzluebbers, 2016; Mac Bean et al., 2020) was probably due to differences in soil and climatic conditions among regions, type of crops grown, and management practices.

3.2 | Relationship between CO_2 flush and soil properties

3.2.1 | Soil physical properties

The 1dC was positively correlated to 4 out of 23 soil physical properties including water-stable aggregate and negatively to a single physical property including bulk density in Froid (one weakly [$r < .50$] and three strongly [$r = .50$–$.80$] related) (Table 3). Similarly, 4dC was positively correlated to 7 out of 23 and negatively to 2 out of 23 physical properties (one weakly and eight strongly related).

In Sidney, 1dC was positively correlated to 6 out of 23 physical properties including aggregate stability and volumetric water content and negatively to 4 out of 23 physical properties, including bulk density, and repacked core available water holding capacity (five weakly and five strongly related) (Table 4). The 4dC was positively correlated to five out of seven and negatively to two out of seven physical properties (four weakly and three strongly related).

The positive correlation between 1dC, 4dC, and soil aggregation and stability in Froid and Sidney suggests that enhanced soil aggregation can stimulate CO_2 evolution probably by increasing microbial growth. Fungi are increasingly

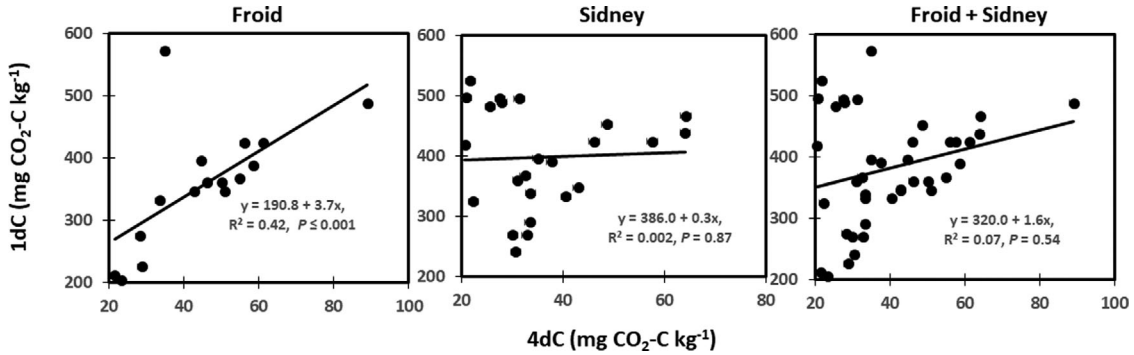

FIGURE 1 Relationship between soil CO_2 flushes at 1- and 4-d incubations (1dC and 4dC, respectively) in Froid, Sidney, and a combination of Froid and Sidney, MT

TABLE 3 Correlation (*r*) between soil CO_2 flushes at 1- and 4-d incubations (1dC and 4dC, respectively) and physical properties in Froid, MT ($n = 16$)

	CO_2 flush			
	1dC		4dC	
Parameter	***r***	***P* value**	***r***	***P* value**
Sand, g kg^{-1}	−.26	.338	−.37	.159
Silt, g kg^{-1}	.21	.439	.33	.212
Clay, g kg^{-1}	.44	.090	.45	.084
Aggregate stability, g kg^{-1}	.45	.079	.44	.086
Water-stable aggregate, g kg^{-1}	**.70**	**.002**	**.64**	**.008**
Dry soil stability index	−.16	.536	−.21	.443
Wet soil stability index	**.55**	**.026**	**.50**	**.048**
Average slake aggregate	**.62**	**.011**	**.53**	**.024**
Stone mass, g	−.14	.603	.02	.948
Stone volume, cm^3	−.20	.458	−.09	.753
Bulk density, Mg m^{-3}	**−.49**	**.050**	**−.67**	**.004**
Macroporosity, cm^3	.38	.149	.21	.426
Mesoporosity, cm^3	−.24	.360	**−.57**	**.043**
Total shrinkage, cm^3 cm^{-3}	.28	.296	.19	.481
Volumetric water content in the field-moist soil, cm^3 cm^{-3}	.42	.101	.36	.177
Volumetric water content at water saturation, cm^3 cm^{-3}	.28	.290	**.56**	**.024**
Volumetric water content at 0.3 kPa, cm^3 cm^{-3}	.27	.310	**.59**	**.016**
Volumetric water content at 10 kPa, cm^3 cm^{-3}	.06	.822	.02	.937
Volumetric water content at 33 kPa, cm^3 cm^{-3}	.37	.154	**.73**	**.001**
Volumetric water content at 1,500 kPa (cm^3 cm^{-3})	.36	.159	.46	.070
Repacked core available water-holding capacity, cm^3 cm^{-3}	.03	.936	**.58**	**.018**
Intact core available water-holding capacity, cm^3 cm^{-3}	.31	.234	−.05	.840
Saturated hydraulic conductivity, cm h^{-1}	**.53**	**.032**	.31	.205

Note. Significant values are shown in bold.

TABLE 4 Correlation (r) between soil CO_2 flushes at 1- and 4-d incubations (1dC and 4dC, respectively) and physical properties in Sidney, MT ($n = 24$)

	CO_2 flush			
	1dC		4dC	
Parameter	***r***	***P* value**	***r***	***P* value**
Sand, g kg^{-1}	.13	.538	**.53**	**.008**
Silt, g kg^{-1}	−.01	.992	**−.52**	**.010**
Clay, g kg^{-1}	−.27	.199	**−.44**	**.030**
Aggregate stability, g kg^{-1}	**.57**	**.004**	**.45**	**.026**
Water-stable aggregate, g kg^{-1}	.19	.367	.39	.060
Dry soil stability index	.14	.515	−.28	.184
Wet soil stability index	**.62**	**.013**	.37	.078
Average slake aggregate	**.68**	**.003**	.16	.461
Stone mass, g	−.22	.303	.29	.166
Stone volume, cm^3	−.24	.252	.27	.204
Bulk density, Mg m^{-3}	**−.42**	**.043**	−.13	.282
Macroporosity, cm^3	**.57**	**.004**	.05	.807
Mesoporosity, cm^3	.19	.371	**.48**	**.018**
Total shrinkage, cm^3 cm^{-3}	**.44**	**.031**	.13	.548
Volumetric water content in the field-moist soil, cm^3 cm^{-3}	**−.42**	**.039**	.11	.602
Volumetric water content at water saturation, cm^3 cm^{-3}	**.51**	**.011**	.19	.373
Volumetric water content at 0.3 kPa, cm^3 cm^{-3}	.22	.304	.32	.121
Volumetric water content at 10 kPa, cm^3 cm^{-3}	−.05	.813	**.41**	**.044**
Volumetric water content at 33 kPa, cm^3 cm^{-3}	**−.42**	**.048**	**.64**	**.007**
Volumetric water content at 1,500 kPa, cm^3 cm^{-3}	−.32	.127	−.39	.061
Repacked core available water-holding capacity, cm^3 cm^{-3}	**−.42**	**.039**	−.35	.089
Intact core available water-holding capacity, cm^3 cm^{-3}	.25	.239	−.32	.122
Saturated hydraulic conductivity, cm h^{-1}	−.11	.602	.12	.601

Note. Significant values are shown in bold.

responsible for soil aggregation compared with bacteria, colonize mainly on the outer portion of aggregates, and mineralize C more than other soil microorganisms, as they constitute >50% of microbial biomass (Hattori et al., 1976; Paul & van Vean, 1978). Several researchers (Liebig et al., 1995; van Es & Karlen, 2019) also reported positive correlation between CO_2 flush and soil aggregation. Similarly, the positive correlation between 1dC, 4dC, volumetric water content, and repacked available water-holding capacity indicates that microbial activity increased as soil water content increased. Franzluebbers (1999) and Liebig et al. (1995) reported that CO_2 flush increased as water-filled pore space increased from 0.53 to 0.73 m^3 m^{-3}. Although Franzluebbers (1999) and van Es and Karlen (2019) determined CO_2 flush using the alkali trap method, Liebig et al. (1995) measured the flush using a gas chromatograph. Similarly, Harris (1981) observed that CO_2 flush increased as soil water content increased from −1,500 to −15 kPa, but decreased at water saturation due to anaerobic condition and limitation of O_2.

The negative correlation between 1dC, 4dC, and bulk density, however, indicates that soil respiration is reduced as soil is compacted due to increased bulk density and decreased porosity. Some researchers (Franzluebbers, 1999; Liebig et al., 1995) found that CO_2 flush decreased as bulk density increased. Torbert and Wood (1992) reported that soil respiration is decreased as bulk density increased from 1.4 to 1.8 Mg m^{-3} at 60% water-filled pore space. They found that soils with lower bulk density had higher proportion of large and small pores, greater pore continuity, and lower surface area that enhanced soil respiration compared with soils with higher bulk density. The positive correlation between 1dC, 4dC, and macro- and mesoporosity in Sidney also suggest that soils with enhanced porosity can enhance CO_2 flush. Soil particle size had variable correlation with 4dC in Sidney whose reasons were not known. The 1dC was correlated to saturated hydraulic conductivity only in Froid, suggesting increased soil respiration with improved water percolation, a case similar to that found by Liebig et al. (1995).

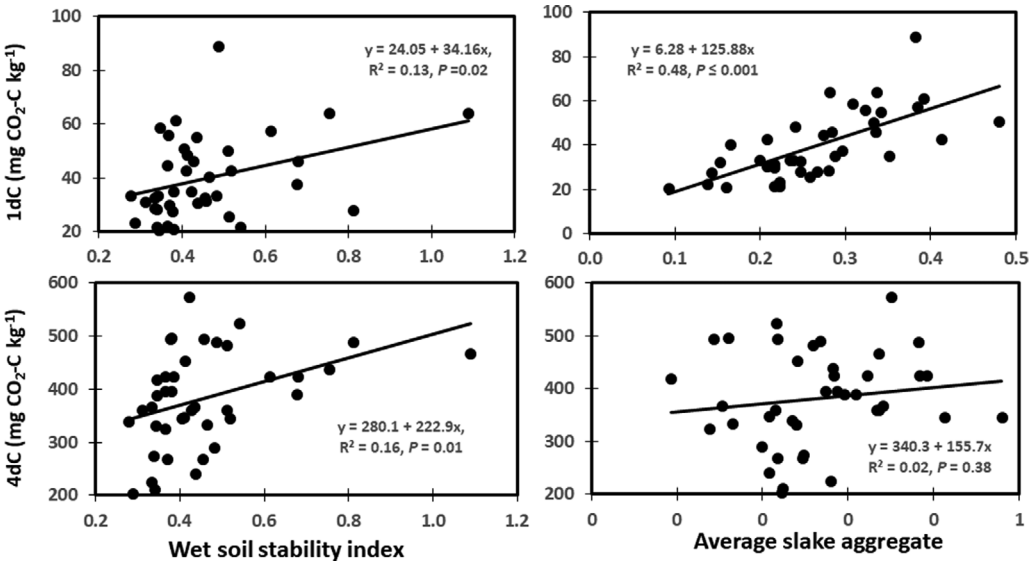

FIGURE 2 Relationships between soil CO_2 flushes at 1- and 4-d incubations (1dC and 4dC, respectively) and wet soil stability index and average slake aggregates for the combined data of Froid and Sidney, MT

Combining the most promising soil physical properties that correlated to 1dC and 4dC from both sites, 1dC and 4dC were linearly related to wet soil stability index (Figure 2). An increase in wet soil stability index by 0.1 increased 1dC and 4dC by 34 and 223 mg CO_2–C kg^{-1}, respectively. Similarly, 1dC was linearly related to average slake aggregate, but there was no relationship between 4dC and average slake aggregate. The mean and standard deviation for these parameters for each treatment in each site are shown in Table 5. Continuous cropping systems enhanced wet soil stability index and aggregate stability, but crop–fallow reduced them, probably due to reduced crop residue input in Froid. In Sidney, cropping system × N fertilization interaction was not significant for these parameters. Wet soil stability index indicates stability of aggregates due to water erosion and average slake aggregates indicates aggregate stability due to slaking. Enhancement of these properties can increase CO_2 flush probably due to entanglement of soil particles by fungal growth that promotes soil aggregation.

3.2.2 | Soil chemical properties

In Froid, 1dC was positively correlated to 7 out of 24 soil chemical properties, including electrical conductivity and B, Ba, Ca, Cd, K, and Pb concentrations (six strongly and one very strongly related) (Table 6). The 4dC was positively correlated to 8 out of 24 chemical properties including electrical conductivity and nutrients (seven strongly and one very strongly related). In Sidney, 1dC was positively correlated to 5 out of 24 chemical properties including Cd, Fe, inorganic and organic P, and Zn concentrations, but negatively to 8 out of 24 chemical properties including pH, electrical conductivity, and nutrients (six weakly and seven strongly related) (Table 7). The 4dC was positively correlated to 10 out of 24 chemical properties including pH, electrical conductivity, and nutrients, but negatively to 1 out of 24 chemical properties, including Al concentration (two weakly and nine strongly related).

The positive correlation between electrical conductivity and 1dC and 4dC at both sites suggests that increased salt concentration probably enhanced CO_2 evolution by increasing microbial activity. As salt is composed of mostly basic cations, increased electrical conductivity also may be linked to increased pH, as is the case with positive correlation between 4dC and pH in Sidney (Table 7). Relationships between 1dC, 4dC, and electrical conductivity for combined data from Froid and Sidney sites revealed that electrical conductivity was marginally ($P = .08$) related to 1dC and linearly related to 4dC (Figure 3). The electrical conductivity accounted for 14–39% variability in 1dC and 4dC, respectively. The mean and standard deviation for this parameter for each treatment in each site are shown in Table 5. Allen et al. (2011) observed that soil respiration was related to electrical conductivity and soil pH that affected microbial activity.

As with electrical conductivity, the positive correlation between 1dC, 4dC, and most nutrients at both sites suggests that increased microbial activity increased the availability of nutrients by mineralizing soil organic matter and amendments. van Es and Karlen (2019) found that 4dC was related to soil pH and P, K, Mg, and Mn concentrations. Negative correlations, however, occurred between 1dC, 4dC, and some micronutrients in Sidney. Nitrogen fertilization has a negative effect on nutrient concentrations compared with no N fertilization (Sainju & Alasinrin, 2020), which also may have

TABLE 5 Mean (± standard deviation) of wet soil stability index (WSSI), average slake aggregate (ASS), electrical conductivity (EC), soil organic matter (SOM), microbially active C (MAC), potentially mineralizable N (PMN), β-glucosidase (BG), and autoclaved citrate-extractable protein (ACEP) as affected by tillage and cropping system combination and N fertilization in Froid and Sidney, MT, for values shown for Figures 2–5

Location	Tillage & cropping system[a]	N fertilization	WSSI	ASS	EC	SOM	MAC	PMN	BG	ACEP
		kg N ha^{-1}			dS m^{-1}	g kg^{-1}	mg C kg^{-1}	mg N kg^{-1}	mg pNP[b] kg^{-1} h^{-1}	mg g^{-1}
Froid	FSTCW		0.42 (± 0.08)ab	0.33 (± 0.06)ab	0.13 (± 0.03)	1.95 (± 0.06)a	353 (± 96)a	25.9 (± 4.5)a	23.3 (± 1.6)a	5.1 (± 0.5)a
	NTCW		0.45 (± 0.06)a	0.41 (± 0.07)a	0.13 (± 0.05)	2.00 (± 0.26)a	364 (± 52)a	19.4 (± 1.8)ab	17.7 (± 5.6)b	5.0 (± 0.5)a
	NTWP		0.33 (± 0.05)b	0.28 (± 0.04)b	0.14 (± 0.05)	1.75 (± 0.50)a	417 (± 140)a	28.0 (± 15.9)a	21.1 (± 8.6)ab	4.1 (± 0.5)ab
	STWF		0.34 (± 0.06)b	0.25 (± 0.03)b	0.10 (± 0.04)	1.30 (± 0.14)b	244 (± 33)b	11.1 (± 2.8)b	11.9 (± 7.3)c	3.1 (± 0.2)b
Sidney	CTWF	0	0.34 (± 0.04)	0.17 (± 0.03)	0.17 (± 0.08)	2.37 (± 0.12)	284 (± 24)	31.6 (± 6.7)	18.7 (± 3.7)	3.1 (± 0.5)
		80/100[c]	0.33 (± 0.04)	0.15 (± 0.07)	0.17 (± 0.03)	2.43 (± 0.06)	267 (± 80)	32.7 (± 5.5)	19.9 (± 5.8)	3.3 (± 0.8)
	NTCW	0	0.88 (± 0.18)	0.30 (± 0.04)	0.17 (± 0.06)	2.57 (± 0.15)	444 (± 157)	46.3 (± 9.4)	26.4 (± 10.1)	3.5 (± 0.9)
		80/100	0.65 (± 0.04)	0.32 (± 0.05)	0.13 (± 0.03)	2.57 (± 0.21)	407 (± 66)	45.9 (± 6.0)	27.4 (± 9.9)	3.7 (± 0.8)
	NTWF	0	0.45 (± 0.08)	0.24 (± 0.04)	0.20 (± 0.01)	2.37 (± 0.06)	301 (± 59)	29.5 (± 5.8)	18.2 (± 6.7)	2.7 (± 0.2)
		80/100	0.41 (± 0.06)	0.19 (± 0.04)	0.22 (± 0.12)	2.37 (± 0.06)	302 (± 48)	22.8 (± 9.5)	20.9 (± 3.9)	3.1 (± 0.5)
	NTWP	0	0.46 (± 0.01)	0.21 (± 0.05)	0.13 (± 0.03)	2.50 (± 0.17)	341 (± 45)	33.6 (± 4.8)	22.7 (± 6.9)	3.4 (± 0.6)
		80/100	0.44 (± 0.06)	0.24 (± 0.03)	0.17 (± 0.03)	2.60 (± 0.10)	365 (± 93)	32.2 (± 9.4)	16.8 (± 2.5)	3.4 (± 0.7)

[a]Tillage and cropping systems in Froid, MT, are fall and spring till continuous spring wheat (FSTCW), no-till continuous spring wheat (NTCW), no-till barley–pea (1984–1999) replaced by spring wheat–pea (2000–2019) (NTWP), and spring till spring wheat–fallow (STWF). Tillage and cropping systems in Sidney, MT, are conventional till barley/spring wheat–fallow (CTWF), no-till continuous barley/spring wheat (NTCW), no-till barley/spring wheat–fallow (NTWF), and no-till barley/spring wheat–pea (NTWP).

[b]pNP, *p*-nitro-phenol.

[c]Numbers followed by different letters within a column in a site are significantly different at $P \leq .05$ by the least square means test.

[d]Nitrogen fertilizer was applied at 80 kg N ha^{-1} to barley from 2006 to 2011 and at 100 kg N ha^{-1} to spring wheat from 2012 to 2019.

TABLE 6 Correlation (r) between soil CO_2 flushes at 1- and 4-d incubations (1dC and 4dC, respectively) and chemical properties in Froid, MT ($n = 16$)

	CO_2 flush			
	1dC		4dC	
Parameter	*r*	*P* value	*r*	*P* value
pH	.47	.066	.17	.523
Buffer pH	.33	.208	.20	.469
Electrical conductivity, dS m^{-1}	**.85**	**<.001**	**.78**	**<.001**
Al concentration, mg kg^{-1}	.19	.484	.27	.301
As concentration, mg kg^{-1}	**.55**	**.032**	**.54**	**.033**
B concentration, mg kg^{-1}	**.79**	**<.001**	.48	.062
Ba concentration, mg kg^{-1}	.21	.438	.02	.150
Ca concentration, mg kg^{-1}	**.63**	**.009**	.34	.198
Cd concentration, mg kg^{-1}	**.52**	**.037**	**.64**	**.008**
Co concentration, mg kg^{-1}	.33	.215	**.55**	**.028**
Cr concentration, mg kg^{-1}	.22	.422	.02	.928
Cu concentration, mg kg^{-1}	.27	.307	.09	.730
Fe concentration, mg kg^{-1}	.10	.703	.33	.210
K concentration, mg kg^{-1}	**.70**	**.003**	**.78**	**<.001**
Mg concentration, mg kg^{-1}	.43	.096	.22	.409
Mn concentration, mg kg^{-1}	.39	.134	**.74**	**.001**
Na concentration, mg kg^{-1}	.28	.299	.15	.587
Na absorption ratio	−.27	.315	−.04	.897
Ni concentration, mg kg^{-1}	.36	.170	.31	.240
P concentration, mg kg^{-1}	.42	.103	.40	.122
Organic P concentration, mg kg^{-1}	.09	.744	−.08	.770
Pb concentration, mg kg^{-1}	**.66**	**.005**	**.86**	**<.001**
S concentration, mg kg^{-1}	−.01	.968	−.08	.768
Zn concentration, mg kg^{-1}	.44	.086	**.64**	**.007**

Note. Significant values are shown in bold.

resulted in negative correlation between CO_2 flush and some nutrients in Sidney.

3.2.3 | Soil biological and biochemical properties

Positive correlation occurred between 1dC and 12 out of 16 soil biological and biochemical properties, including total C and N concentrations, soil organic matter, water-extractable organic N, total N, and organic C, $KMnO_4$-extractable C, microbially active C, β-glucosidase, ACEP, and potentially mineralizable N in Froid (10 strongly and 2 very strongly related) (Table 8). Similarly, positive correlation occurred between 4dC and 11 out of 16 biological and biochemical properties (10 strongly and 1 very strongly related).

In Sidney, positive correlation occurred between 1dC and 8 out of 15 soil biological and biochemical properties, including soil organic matter, water-extractable organic N and C, microbially active C, β-glucosidase, ACEP, potentially mineralizable N, and N-acetyl-β-glucosaminidase (NABG), but negative correlation between 1dC and 1 out of 15 properties including NO_3–N concentration (eight strongly and one very strongly related) (Table 9). Similarly, positive correlation occurred between 4dC and 1 out of 15 biological and biochemical properties, including potentially mineralizable N, but negative correlation between 4dC and 1 out of15 properties, including water-extractable total N (both are weakly related).

Most of the positive correlations among 1dC, 4dC, and soil biological and biochemical parameters in Froid and Sidney, except for the correlation between the 4dC and the parameters in Sidney, suggest that CO_2 flush is an indicator of substrate availability, N mineralization, and enzyme activity. Although C and N substrates availability provide energy and food for microbes, enhanced enzyme activity can increase the decomposition of soil organic matter and amendments, affecting nutrient availability, C sequestration, leaching loss, and

TABLE 7 Correlation (r) between soil CO_2 flushes at 1- and 4-d incubations (1dC and 4dC, respectively) and chemical properties in Sidney, MT ($n = 24$)

	CO_2 flush			
	1dC		4dC	
Parameter	***r***	***P* value**	***r***	***P* value**
pH	**–.46**	**.022**	**.56**	**.005**
Buffer pH	.19	.371	**.67**	**<.001**
Electrical conductivity, dS m^{-1}	**–.59**	**.002**	**.50**	**.012**
Al concentration, mg kg^{-1}	.33	.116	**–.68**	**.003**
As concentration, mg kg^{-1}	**–.43**	**.037**	**.72**	**<.001**
B concentration, mg kg^{-1}	–.31	.152	**.53**	**.008**
Ba concentration, mg kg^{-1}	–.20	.342	**–.15**	.477
Ca concentration, mg kg^{-1}	**–.57**	**.003**	**.59**	**.003**
Cd concentration, mg kg^{-1}	**.58**	**.003**	**–.16**	.455
Co concentration, mg kg^{-1}	**–.44**	**.032**	**.46**	**.023**
Cr concentration, mg kg^{-1}	.19	.382	**–.17**	.426
Cu concentration, mg kg^{-1}	**–.57**	**.003**	.39	.059
Fe concentration, mg kg^{-1}	**.54**	**.006**	**.51**	**.012**
K concentration, mg kg^{-1}	.10	.632	.25	.235
Mg concentration, mg kg^{-1}	**–.44**	**.032**	**–.24**	.252
Mn concentration, mg kg^{-1}	**–.46**	**.024**	**.49**	**.016**
Na concentration, mg kg^{-1}	–.30	.150	**–.15**	.471
Na absorption ratio	–.27	.204	**–.28**	.197
Ni concentration, mg kg^{-1}	–.25	.222	**–.10**	.628
P concentration, mg kg^{-1}	**.77**	**<.001**	.18	.399
Organic P concentration, mg kg^{-1}	**.45**	**.028**	.19	.369
Pb concentration, mg kg^{-1}	–.30	.158	**.57**	**.004**
S concentration, mg kg^{-1}	.13	.537	**–.37**	.071
Zn concentration, mg kg^{-1}	**.50**	**.013**	.14	.510

Note. Significant values are shown in bold.

greenhouse gas emissions. Some researchers (Franzluebbers, 2016; Franzluebbers et al., 2000; Haney et al., 2010) reported that CO_2 flushes at 1- to 3-d incubations were related to the flush at 24-d incubation, microbial biomass, N mineralization, and water-extractable C and N. Others (Wang et al., 2003) found that CO_2 flush at 7-d incubation was related to soil organic matter, water-soluble C, and K_2SO_4– and $KMnO_4$–extractable C, suggesting that soil respiration was mostly affected by C substrate availability rather than microbial biomass. van Es and Karlen (2019) found that 4dC was correlated to soil organic matter, $KMnO_4$–extractable C, and ACEP.

When regression analysis was conducted for 1dC and 4dC with most promising biological and biochemical indicators for combined data in both sites, soil organic matter, microbially active C, and potentially mineralizable N were linearly related to the 1dC (Figure 4). Soil organic matter and potentially mineralizable N were nonlinearly related to 4dC. Similarly, β-glucosidase was linearly related to 1dC and ACEP was linearly and nonlinearly related to 1dC and 4dC, respectively (Figure 5). The fraction of variation explained by soil biological and biochemical properties in 1dC and 4dC ranged from 0.02 to 0.85. The mean and standard deviation for these parameters for each treatment in each site are shown in Table 5. Increased crop residue input enhanced soil biological and biochemical parameters with continuous cropping, but crop–fallow reduced them in Froid. In Sidney, the interaction of cropping system × N fertilization was not significant for these parameters.

One of the major benefits of determining CO_2 flush in short-term incubation is estimating potentially mineralizable N, which takes long time to measure. As a result, potentially mineralizable N of soils is often ignored while recommending N fertilization rates to crops. This can lead to excessive accumulation of soil residual N after crop harvest that can degrade the environment by enhancing N leaching in the surface- and groundwater and greenhouse gas emissions. The 1dC and 4dC were strongly to very strongly correlated with potentially

TABLE 8 Correlation (r) between soil CO_2 flushes at 1- and 4-d incubations (1dC and 4dC, respectively) and biological and biochemical properties in Froid, MT ($n = 16$)

	CO_2 flush			
	1dC		4dC	
Parameter	***r***	***P* value**	***r***	***P* value**
Total C, g C kg^{-1}	**.58**	**.018**	**.71**	**.002**
Soil organic matter, g kg^{-1}	**.78**	**<.001**	**.77**	**<.001**
Total N, g N kg^{-1}	**.56**	**.023**	**.70**	**.002**
Water-extractable organic N, mg N kg^{-1}	**.57**	**.022**	.47	.066
Water-extractable total N, mg N kg^{-1}	**.71**	**.002**	**.65**	**.007**
NH_4–N, mg N kg^{-1}	.35	.181	.14	.601
NO_3–N, mg N kg^{-1}	.49	.054	.40	.116
$KMnO_4$ extractable C, mg C kg^{-1}	**.67**	**.004**	**.89**	**<.001**
Water-extractable C, mg C kg^{-1}	**.71**	**.002**	**.76**	**<.001**
Microbially active C, mg mg^{-1}	**.96**	**<.001**	**.56**	**.025**
β-glucosidase, mg pNP[a] kg^{-1} h^{-1}	**.65**	**.006**	**.64**	**.008**
ACEP, mg protein g^{-1}[b]	**.52**	**.039**	**.71**	**.002**
Potentially mineralizable N, mg N kg^{-1}	**.88**	**<.001**	**.79**	**<.001**
NABG, mg pNP kg^{-1} h^{-1}[a,c]	.30	.259	.07	.650
Arysulfatase, mg pNP kg^{-1} h^{-1}	.44	.090	.18	.510
Phosphomonoesterase, mg pNP kg^{-1} h^{-1}	.42	.110	**.52**	**.037**

Note. Significant values are shown in bold.
[a]pNP, *p*-nitro-phenol.
[b]ACEP, autoclaved citrate extractable protein.
[c]NABG, N-acetyl β-glucosaminidase.

TABLE 9 Correlation (r) between soil CO_2 flushes at 1- and 4-d incubations (1dC and 4dC, respectively) and biological and biochemical properties in Sidney, MT ($n = 24$)

	CO_2 flush			
	1dC		4dC incubation	
Parameter	***r***	***P* value**	***r***	***P* value**
Total C, g C kg^{-1}	.39	.056	.15	.473
Soil organic matter, g kg^{-1}	**.56**	**.004**	.23	.274
Total N, g N kg^{-1}	.17	.426	.18	.397
Water extractable organic N, mg N kg^{-1}	**.52**	**.009**	−.21	.327
Water extractable total N, mg N kg^{-1}	−.27	.203	**−.46**	**.024**
NH_4–N, mg N kg^{-1}	.10	.636	−.19	.384
NO_3–N, mg N kg^{-1}	**−.50**	**.012**	−.36	.082
$KMnO_4$ extractable C, mg C kg^{-1}	.31	.145	.35	.090
Water-extractable C, mg C kg^{-1}	**.74**	**<.001**	.13	.862
Microbially active C, mg mg^{-1}	**.97**	**<.001**	−.04	.546
β-glucosidase, mg pNP[a] kg^{-1} h^{-1}	**.68**	**<.001**	−.09	.689
ACEP, mg protein g^{-1}[b]	**.69**	**<.001**	−.32	.119
Potentially mineralizable N, mg N kg^{-1}	**.67**	**<.001**	**.42**	**.039**
NABG, mg pNP kg^{-1} h^{-1}[c]	**.66**	**<.001**	.28	.186
Arysulfatase, mg pNP kg^{-1} h^{-1}	−.02	.930	.32	.125

Note. Significant values are shown in bold.
[a]pNP, *p*-nitro-phenol.
[b]ACEP, autoclaved citrate extractable protein.
[c]NABG, N-acetyl β-glucosaminidase.

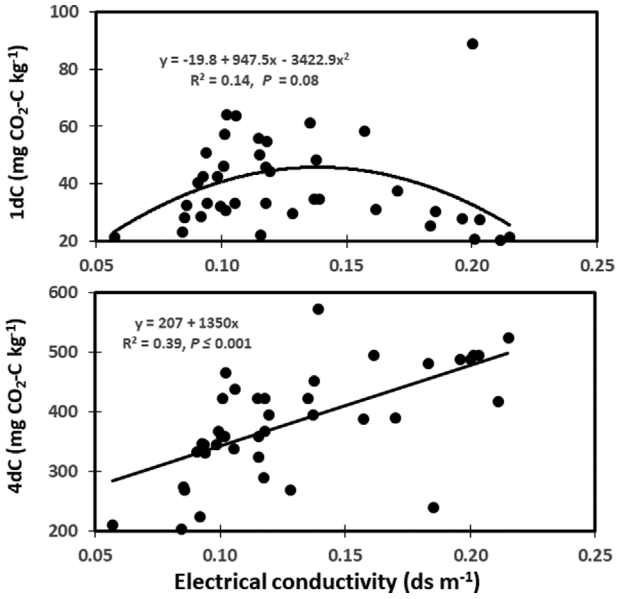

FIGURE 3 Relationships between soil CO_2 flushes at 1- and 4-d incubations (1dC and 4dC, respectively) and electrical conductivity for the combined data of Froid and Sidney, MT

mineralizable N in Froid (Figure 4) and weakly to strongly related in Sidney (Tables 8 and 9). This indicates that the relationships between 1dC, 4dC, and potentially mineralizable N depend on soil texture, duration of the experiment, and cropping systems among sites. Coarse-textured soil and longer duration of the experiment showed stronger relationships between 1dC, 4dC, and potentially mineralizable N in Froid than fine-textured soil and shorter duration of the experiment in Sidney. The potentially mineralizable N can be estimated by determining CO_2 flush during short-term incubations and can be used to reduce N fertilization rates to crops, thereby reducing environmental degradation (Castellanos & Pratts, 1981; Haney et al., 2001; Franzluebbers, 2016).

3.3 | Relationship between CO_2 flush and crop yield

The 1dC was nonlinearly related to mean annualized crop yield across years in Froid, Sidney, and a combination of both sites ($P = .07$) (Figure 6). The 1dC accounted for 63% of variability in crop yield in Froid, 31% in Sidney, and 13% for the combined sites. Similarly, 4dC was nonlinearly related to mean annualized crop yield in Froid, comprising 43% of variability in crop yield, but not in Sidney and the combined sites. The extent of relationship as determined by R^2 values varied from .13 to .63, with a mean value of .25. The mean and standard deviation of mean annualized crop yield across years for both sites are shown in Table 10. In Froid, continuous cropping increased mean annualized crop yield with FSTCW and NTCW compared with STWF where absence of crops during the fallow period decreased the yield (Table 10). Reduced yield of pea compared with spring wheat also reduced mean annualized crop yield with NTWP compared with FSTCW and NTCW. In Sidney, continuous cropping with N fertilization increased mean annualized crop yield in NTCW with 80/100 kg N ha^{-1} compared with other treatments, except NTWP with 80/100 kg N ha^{-1} (Table 10). Absence of crops during the fallow period and lack of N fertilization reduced yields in other treatments.

Our results of significant relationship between 1dC, 4dC, and crop yields were similar to those observed by several researchers (Alves de Castro Lopes et al., 2013; van Es & Karlen, 2019; Yost et al., 2018). Several researchers (Haney et al., 2001; Franzluebbers, 2016) also reported that CO_2 flushes at 1- to 3-d incubations were related to forage N uptake. Others (Mac Bean et al., 2020; Roper et al., 2017) found that 4dC was not consistently related to crop yield, although significant relationship occurred in years with favorable weather condition. They suggested that the relationship should be examined in regions with particular soil type, climatic condition, and management practice rather than using it for the comprehensive interpretation of soil health indicator for broad regions.

The significant relationship between CO_2 flush as a soil health indicator and crop yield is an important implication for producers, as it shows immediate and direct benefit to them (Franzluebbers, 2016; Mac Bean et al., 2020; van Es & Karlen, 2019). This relationship may not hold true in some years due to non-soil factors, such as reduction in crop yields due to climatic conditions (e.g., droughts, flood, and natural calamities) and crop damage by high weed pressure or pest infections. As a result, the relationship should be examined in long-term experiments where long-term data for crop yields are available and mean yield across years can be used (Alves de Castro Lopes et al., 2013; Congreves et al., 2015; Roper et al., 2017; van Es & Karlen, 2019). Relationships between CO_2 flush and other soil properties showing indirect benefits, such as improvements in soil and environmental quality, whose economic benefits are difficult to ascertain, may be less important to producers (Franzluebbers; Mac Bean et al., 2020; van Es & Karlen, 2019).

3.4 | Comparison between CO_2 flushes at one- and four-day incubations

The 1dC was affected by treatments in both Froid and Sidney, but the treatment effect on 4dC occurred only in Froid (Tables 1 and 2). Average 1dC across treatments was 9 mg CO_2–C kg^{-1} greater in Froid than Sidney, but the average 4dC was 40 mg CO_2–C kg^{-1} greater in Sidney than Froid. Although the coarse-textured soil in Froid had slightly greater

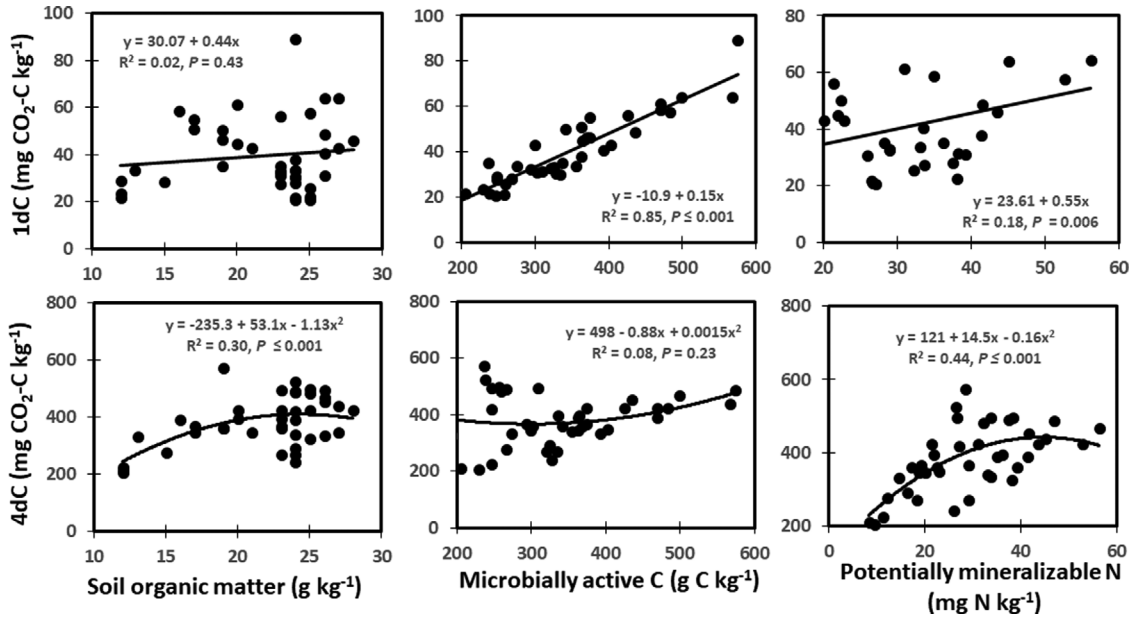

FIGURE 4 Relationships between soil CO_2 flushes at 1- and 4-d incubations (1dC and 4dC, respectively) and soil organic matter, microbially active C, and potentially mineralizable N for the combined data of Froid and Sidney, MT

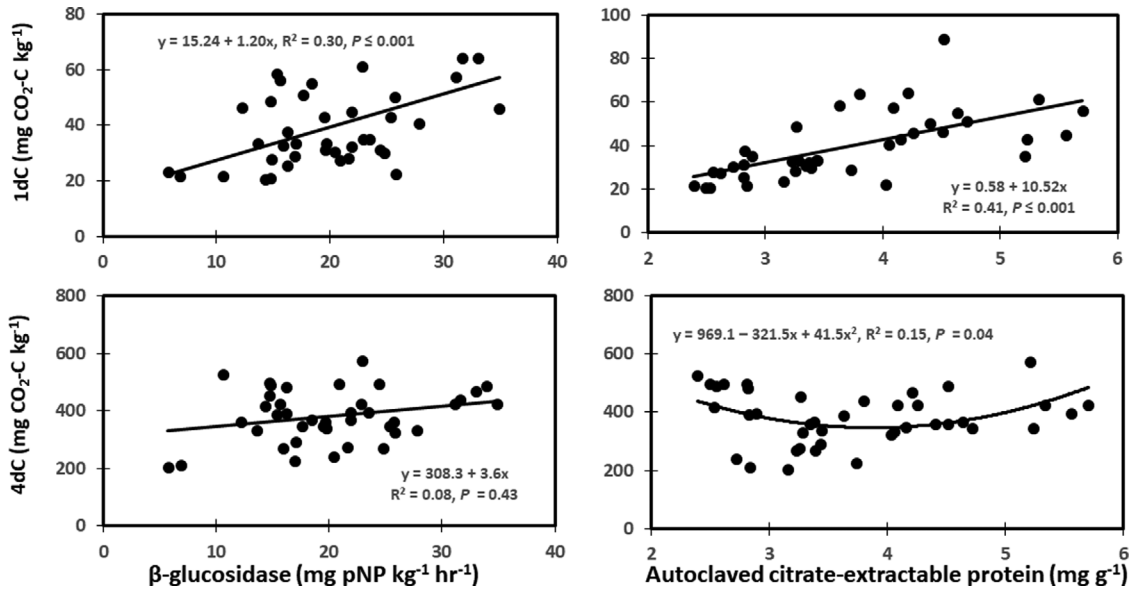

FIGURE 5 Relationships between soil CO_2 flushes at 1- and 4-d incubations (1dC and 4dC, respectively) and β-glucosidase and autoclaved citrate-extractable protein for the combined data of Froid and Sidney, MT. pNP, p-nitro-phenol

1dC than the medium-textured soil in Sidney, this was not the case with 4dC. Longer incubation of medium- than coarse-textured soil produced more CO_2, probably due to increased mineralization of intermediate and nonlabile soil organic matter.

The 4dC correlated with greater number of soil physical parameters than 1dC in Froid, but the trend reversed in Sidney (Tables 3 and 4). As application of chemicals, such as N fertilizer, can degrade soil physical properties (Tilman et al., 2002) and 4dC provides more sensitive results with greater range than 1dC (Mac Bean et al., 2020), our results showed that 1dC and 4dC were equally sensitive to soil physical properties, depending on soil texture, management practices, and the age of the experiment. This is because 1dC and 4dC were similarly related to soil physical properties in Froid and Sidney (Tables 3 and 4).

The number of soil chemical parameters correlated to 1dC and 4dC were 7 and 8, respectively, in Froid (Table 6). In

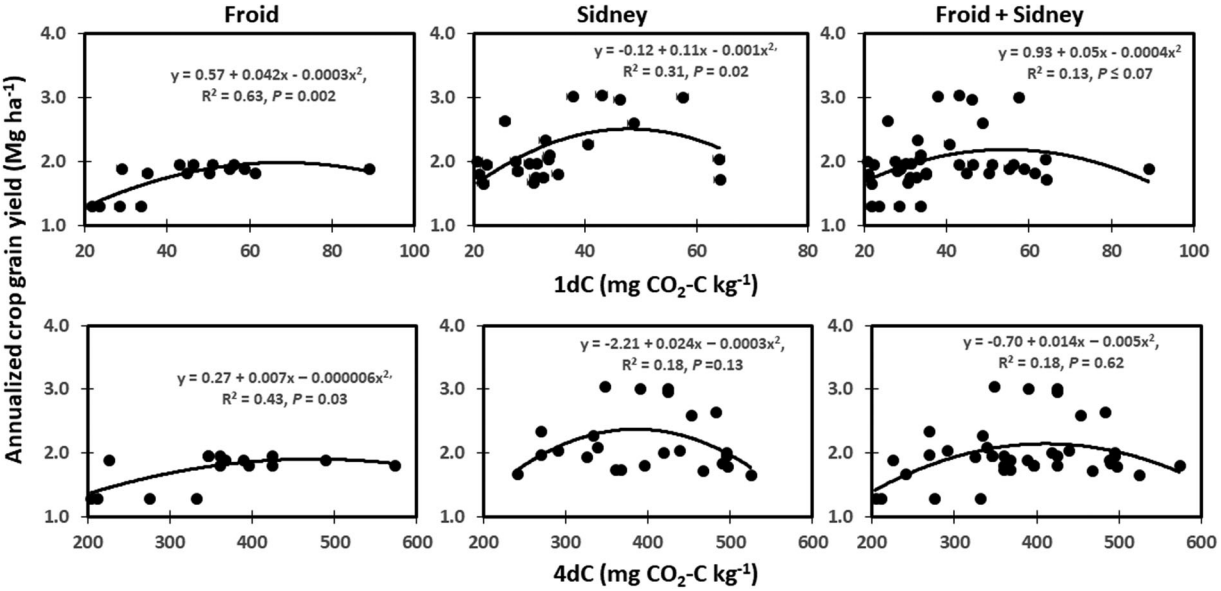

FIGURE 6 Relationships between soil CO_2 flushes at 1- and 4-d incubations (1dC and 4dC, respectively) and mean annualized crop yield across years for Froid, Sidney, and the combined data of Froid and Sidney, MT

TABLE 10 Mean (± standard deviation) annualized crop yield across years as affected by tillage and cropping system combination and N fertilization in Froid and Sidney, MT

Tillage and cropping system[a]	N fertilization	Mean annualized crop yield
	kg N ha^{-1}	Mg ha^{-1}
Froid		
FSTCW		1.77 (± 0.20)a[b]
NTCW		1.85 (± 0.31)a
NTWP		1.47 (± 0.13)b
STWF		1.50 (± 0.15)b
Sidney		
CTWF	0	1.76 (± 0.03)c
	80/100[c]	2.02 (± 0.08)bc
NTCW	0	1.87 (± 0.16)c
	80/100	3.00 (± 0.03)a
NTWF	0	1.72 (± 0.08)c
	80/100	2.01 (± 0.03)bc
NTWP	0	2.19 (± 0.20)bc
	80/100	2.76 (± 0.25)ab

[a]Tillage and cropping systems in Froid, MT, are fall and spring till continuous spring wheat (FSTCW), no-till continuous spring wheat (NTCW), no-till barley–pea (1984–1999) replaced by spring wheat–pea (2000–2019) (NTWP), and spring till spring wheat–fallow (STWF). Tillage and cropping systems in Sidney, MT, are conventional till barley/spring wheat–fallow (CTWF), no-till continuous barley/spring wheat (NTCW), no-till barley/spring wheat–fallow (NTWF), and no-till barley/spring wheat–pea (NTWP).

[b]Numbers followed by different letters within a column are significantly different at $P \leq .05$ by the least square means test.

[c]Nitrogen fertilizer was applied at 80 kg N ha^{-1} to barley from 2006 to 2011 and at 100 kg N ha^{-1} to spring wheat from 2012 to 2019.

contrast, the number of chemical parameters correlated positively or negatively to 1dC and 4dC were 13 and 11, respectively, in Sidney (Table 7). Twelve and eleven soil biological and biochemical properties correlated to 1dC and 4dC, respectively, in Froid (Table 8). In Sidney, nine and two soil biological and biochemical properties correlated to 1dC and 4dC, respectively (Table 9). The 1dC was linearly or nonlinearly related to mean annualized crop yield in Froid, Sidney, and a combination of both sites, but 4dC was nonlinearly related to crop yield only in Froid (Figure 6).

The greater number of correlations of 1dC and 4dC with soil chemical, biological, and biochemical properties in Froid than in Sidney was probably due to differences in the nature of treatments, soil type, and length of the experiment between the sites as well as method of determination. Inclusion of N fertilizer treatment may have disrupted soil properties in Sidney (Karlen et al., 1997; Tilman et al., 2002), resulting in reduced correlations between CO_2 flushes and soil chemical, biological, and biochemical properties in Sidney compared to Froid where N fertilizer treatment was absent. Although C mineralization can be higher in coarse- than fine-textured soils (Sainju, Caesar-Tonthat, & Caesar, 2012), increased changes in soil properties due to management practices can be expected to occur in longer duration of experiment, resulting in increased correlation between CO_2 flush and soil properties. The 1dC was lower with crop–fallow than continuous cropping, regardless of soil texture and duration of the experiment in Froid and Sidney (Tables 1 and 2). We expected to observe even greater difference among treatments with 4dC. However, weaker difference among treatments in Froid or no significant difference in Sidney occurred in 4dC. One probable reason may be the differences in methods used for

determining 1dC and 4dC. The 1dC was determined by using the infrared analyzer and 4dC by using the alkali trap.

These results suggest that 1dC responded better to treatments and was similar to or better than 4dC in predicting the response of the CO_2 flush to soil properties and crop yields. Furthermore, no chemical was used for the determination of 1dC compared with numerous chemicals use in 4dC. Therefore, 1dC using the infrared analyzer can be considered as a rapid, inexpensive, and sensitive soil health indicator that responds to management practices and relates to most soil physical, chemical, and biological properties and crop yields. As a result, it can be used in regular soil testing to measure soil health under dryland cropping systems in the semiarid region of the northern Great Plains.

4 | CONCLUSIONS

The 1dC and 4dC determined by the infrared analyzer and alkali trap, respectively, were variably affected by treatments and related differently to soil physical, chemical, biological, and biochemical properties as well as mean annualized crop yields across years between sites. Crop–fallow reduced CO_2 flushes compared with continuous cropping in Froid and Sidney, MT, except for 4dC in Sidney. The 1dC was similar to or better than 4dC in determining relationships between the CO_2 flush and soil physical, chemical, biological, and biochemical properties and crop yields. More soil parameters were related to CO_2 flushes in Froid than Sidney. The 1dC related to mean annualized crop yield in Froid, Sidney, and a combination of both sites, but 4dC related to crop yield only in Froid. Because of the rapid and inexpensive measurement and increased sensitivity to management practices, soil properties, and crop yields, 1dC using the infrared analyzer can be used as a soil health indicator in routine soil testing for dryland cropping systems in the northern Great Plains.

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AUTHOR CONTRIBUTIONS

Upendra M. Sainju: Conceptualization; Data curation; Formal analysis; Investigation; Project administration; Supervision; Writing-original draft; Writing-review & editing. Daniel Liptzin: Data curation; Funding acquisition; Investigation; Methodology; Resources; Validation. Sadikshya M. Dangi: Data curation; Formal analysis; Investigation; Writing-review & editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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