

Article

Contrasting Freeze–Thaw Regimes and Straw Amendment Reshape Microbial Necromass and Soil Carbon Pools in Saline–Alkali Paddy and Maize Soils

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Abstract

Soil carbon dynamics in seasonal freeze–thaw zones reflect the interplay of two temporal regimes: high-frequency symmetric cycles driven by diurnal temperature fluctuations, and low-frequency asymmetric cycles with extended freezing and brief thawing. Their differential association with microbially derived carbon pools remains unclear. We incubated saline–alkali paddy and maize soils for 45 days under three regimes: a 5 °C control (CK), symmetric freeze–thaw (12 h/12 h at 5 °C/−10 °C; SFT) and asymmetric freeze–thaw (12 h/60 h at 5 °C/−10 °C; ASFT), each with and without straw. Dissolved organic carbon (DOC), microbial biomass carbon (MBC), microbial necromass carbon (MNC), fungal necromass carbon (FNC), bacterial necromass carbon (BNC) and the FNC/BNC ratio were determined at six sampling times. The asymmetric regime depleted the biomass and necromass pools most strongly and accumulated the most DOC; the symmetric regime produced smaller changes. Straw amendment reversed this depletion under the asymmetric regime and raised every necromass pool above its initial value, but it had the opposite effect at constant temperature and only a marginal effect under the symmetric regime. Substrate supply, rather than freezing stress alone, therefore appears to constrain necromass accumulation. The biomass and necromass pools changed in a coordinated way, whereas DOC was uncorrelated with them. Straw amendment may thus offset the suppression of necromass accumulation under the asymmetric freeze–thaw regime. Because the two regimes differ in several cycle components at once, the differences are attributed to the regimes as a whole, and the results describe a 45-day incubation rather than long-term carbon stabilization.

Keywords: soil carbon stabilization; soil management; microbial necromass carbon; freeze–thaw regimes; fungal necromass carbon; bacterial necromass carbon; dissolved organic carbon; straw amendment



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1. Introduction

Seasonal freeze–thaw is an important climatic feature in northern China. Studies have shown that freeze–thaw can destroy the structure of aggregates, change soil microbial activity, affect the migration and transformation of nutrients, and the decomposition of organic matter mediated by microorganisms [1], thus having a great impact on soil carbon and nitrogen cycling [2]. However, the effects of freeze–thaw on soil organic carbon (SOC) in saline–alkali farmland at middle and high latitudes and its mechanism are rarely studied,

in particular the characteristics of exogenous organic carbon on SOC under freeze–thaw action are not very clear.

Farmland ecosystems are the largest artificial ecosystems on Earth, and farmland soil carbon pools are carbon pools that are subject to strong anthropogenic disturbance and can be regulated on short time scales [3,4]. Soil active organic carbon is a kind of SOC that moves quickly, has poor stability, is easy to oxidize, mineralizes, and can directly participate in soil biochemical transformation [5]. Microbial necromass carbon (MNC) plays an important role in maintaining soil stability [6,7], and the key determinants of MNC are microbial properties and soil properties [8]. Dissolved organic carbon (DOC) and microbial biomass carbon (MBC) are both active organic carbon compounds. DOC is sensitive to changes in the regional microenvironment and plays an important role in the carbon cycle and soil nutrient supply [9]. Soil MBC is a key player in nutrient cycling [10], and soil MBC is regarded as an important indicator of soil fertility and biological quality as an unstable reservoir of available nutrients for plants [11]. Straw addition, as a type of exogenous organic carbon, is a common management method in western Jilin [12]. It is of great significance to study the changes in active organic carbon composition to elucidate the stability mechanism of soil organic carbon in saline–alkali farmland under freeze–thaw action.

Currently, there is a heated debate about the potential to increase organic carbon storage in soils, strategies to achieve organic carbon storage, and the actual benefits for agriculture and climate. Some scholars have argued that [13] recalcitrant organic carbon has long been considered to be the main process driving the persistence of organic carbon in soils, and is generally considered to be of microbial origin. Therefore, the term “recalcitrant” should no longer be used to define the global dynamics of molecules in soils; instead, the term “persistence” should be used. In short, the preservation and decomposition of organic carbon should be a process of dynamic equilibrium. Faced with the problem of soil degradation in saline–alkali soil, Gong et al. [14] explored the effects of microbial residues and ecological stoichiometric characteristics of soil organic matter on organic carbon accumulation in saline–alkali grassland through the interaction between biochar and nutrients. Previous studies have shown that paddy straw has a positive effect on soil microbial properties, and crop straw plays a crucial role in soil remediation, especially to reduce the pressure of saline–alkali soil [15]. Recent studies have found that organic–organic interactions mainly drive SOC accumulation [16], suggesting that accurate prediction of SOC dynamics remains challenging. The freeze–thaw cycle is a common phenomenon in seasonal permafrost areas. Existing studies typically simplify seasonal freeze–thaw as a single freeze–thaw pattern; however, under natural conditions, seasonal freeze–thaw zones actually encompass both processes simultaneously: diurnal temperature fluctuations drive high-frequency diurnal freeze–thaw cycles (symmetric type), while climatic transitions drive low-frequency long-freezing–short-thawing cycles (asymmetric type). Nevertheless, no study has yet systematically compared the differential effects of these two freeze–thaw temporal regimes within the same experimental framework, particularly lacking in-depth investigation into “how temporal asymmetry regulates soil carbon pool composition and stability under identical temperature boundary conditions.” Based on the formation pathways and the efficiency of soil organic carbon, this experimental design studied the effects of external organic carbon on organic carbon in saline–alkali land in western Jilin Province under freeze–thaw conditions.

In this paper, the study area in western Jilin Province is not only an important grain-producing area and commercial grain base in China [17], but also one of the three major saline–alkali soil concentration areas in the world [18]. Based on the differences and influencing factors of active organic carbon, combined with the effectiveness of soil nutrients,

the characteristics of soil organic carbon changes in saline–alkali farmland under the influence of freeze–thaw action were analyzed, and the influencing factors were examined from the perspective of soil active carbon. This is intended to help align the management strategy of soda saline–alkali land with the complexity of the soil carbon pool, and to provide theoretical support for the efficient use of saline–alkali farmland. Understanding how exogenous organic carbon modifies soil carbon pools under freeze–thaw conditions is therefore essential for maintaining the carbon-sink function of saline–alkali farmland soils.

We tested three hypotheses. (H1) Because the asymmetric regime combines a longer freezing phase with a shorter thawing phase than the symmetric regime, we hypothesized that it would be associated with a greater reduction in microbial necromass carbon, and particularly in bacterial necromass carbon (BNC), than the symmetric regime (SFT) and the constant-temperature control. (H2) We further hypothesized that this reduction would be accompanied by a shift in the composition of the necromass pool towards a higher fungal-to-bacterial necromass ratio (FNC/BNC). (H3) We further hypothesized that straw amendment would partially buffer these responses by alleviating substrate limitation during thawing periods, with a proportionally larger effect under ASFT than under the SFT or the CK.

2. Materials and Methods

2.1. Study Area Description

Songyuan City is located in the western part of Jilin, and saline–alkali soil accounts for 25–30% of the land resources in the study area (Figure 1). The main anions of saline–alkali soil in the region are CO_3^{2-} and HCO_3^- [19], which can be divided into saline soil, alkaline soil and saline–alkali soil according to the soil-forming process, and the thickness and position of the alkalis layer in the soil profile differ, with a maximum thickness of 80–100 cm. The western part of Jilin has a typical semi-arid and semi-humid continental monsoon climate [20]. The whole growing season of crops is under a mid-temperate monsoon climate, with an average annual temperature of about 4.5 °C and an average annual precipitation of about 400 mm (mean values for 2023–2024). Both rice (*Oryza sativa* L.) and maize (*Zea mays* L.) are the main food crops of Songyuan.

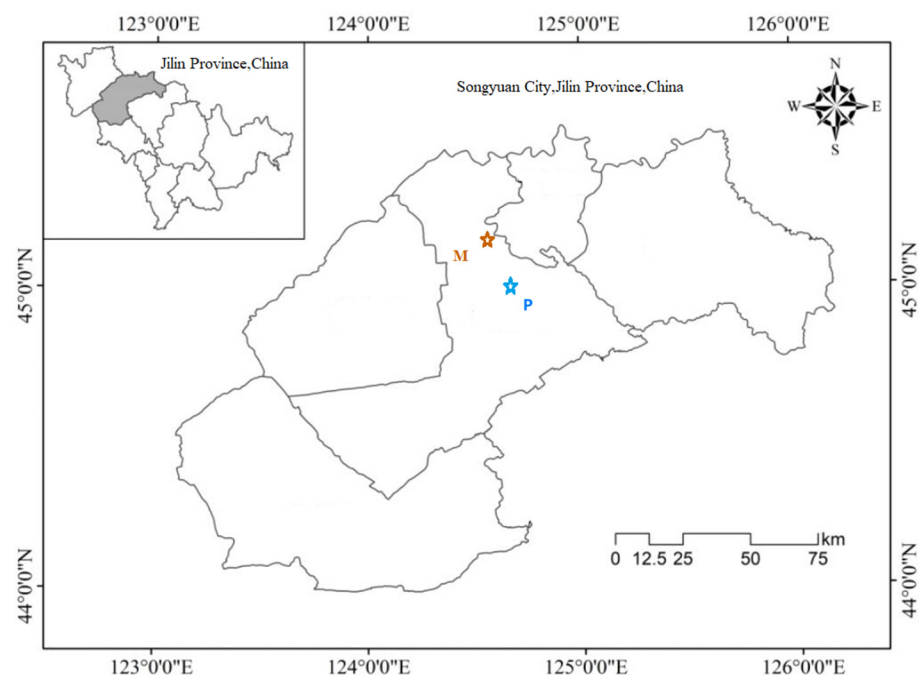


Figure 1. The location of the study area (Songyuan) in Jilin Province, in northeastern China.

Songyuan City has a long winter and a harsh climate, and agriculture practises a one-year-a-crop farming system. Maize fields in the study area are generally sown in early May when the soil is warmed up and harvested from late September to early October. According to the needs of maize growth and development, nitrogen, phosphorus and potassium compound fertilizer and well-rotted organic fertilizer were generally applied at the seedling stage in the study area, and nitrogen fertilizer and compound fertilizer were appropriately supplemented at the jointing stage. Since the corn fields with irrigation conditions do not exceed one-fifth of the total maize planting area, rain-fed and water-saving agriculture is developed in most areas. Paddy is generally transplanted in mid-May and harvested in October. Paddy planting implements the management system of “three irrigation and three rows”, and organic fertilizer and compound fertilizer are applied at the first irrigation, and nitrogen fertilizer and potassium fertilizer are applied at the tillering stage after transplanting.

Paddy field reclamation began as early as 1929, and at the same time as the large-scale reclamation of paddy fields, the area of dry fields was also expanding, and the area of salinized soil in the process of land development was also gradually expanding. In order to cope with the increasingly serious problem of soil salinization, through a series of land consolidation and water conservancy projects, new cultivated land (reclamation of paddy fields) has been realized, and medium- and low-yield fields have been transformed.

2.2. Soil Sampling and Experimental Design

At the end of October 2023, a representative maize field and a representative paddy field were selected in the saline–alkali soil area of western Jilin Province. After removal of surface vegetation and litter, soil was collected from two layers, topsoil (0–20 cm) and subsoil (20–40 cm), at each site by the five-point sampling method. At each site and each depth, the soil from the five points was thoroughly mixed to form a single composite sample, packed into a sealed ziplock bag, and quickly transported back to the laboratory, where it was passed through a 4 mm sieve to remove visible roots and other impurities and fully mixed for the subsequent experiments. Part of each sample was used to determine the background physicochemical properties (Table 1) and the remainder was used for the incubation experiment. The background values of pH, exchangeable sodium percentage (ESP) and soil salt content (SSC) reported in Table 1 were obtained during a preliminary survey of the sampling sites carried out in April 2023; these parameters were used to characterize the degree of salinization and to establish that the two sites were comparable in salinity.

Table 1. Background properties of the two sampling sites, determined in April 2023 on composite samples obtained by the five-point sampling method (mean of three analytical replicates).

Sampling Sites	pH	ESP (%)	SSC (%)	Fertilization Treatment	Experimental Area	Type of Crop
Paddy	8.59	8.08	0.23	N, P and K	98 m × 102 m	Paddy
Maize	8.51	8.19	0.24	N, P and K	95 m × 100 m	Maize

According to the actual freeze–thaw cycle during the freeze–thaw period in the saline–alkali soil area of western Jilin, two contrasting freeze–thaw regimes were established: a symmetric freeze–thaw treatment (SFT; thawing/freezing temperature of 5 °C/−10 °C, thawing/freezing time of 12 h/12 h) and an asymmetric freeze–thaw treatment (ASFT; thawing/freezing temperature of 5 °C/−10 °C, thawing/freezing time of 12 h/60 h). A treatment without freezing (constant 5 °C) served as the control (CK). Each regime was combined with and without straw amendment, giving six treatments that were applied to

both the topsoil and the subsoil of the two soils. Straw was matched to the soil of origin: rice straw was applied to the paddy soil and maize straw to the maize soil.

The specific indoor incubation method was as follows: 40 g of fresh soil was weighed into a 50 mL sample bottle, the water content was adjusted to 25%, the mouth of the bottle was sealed with plastic wrap leaving a ventilation hole, and the bottle was placed in a 5 °C constant-temperature incubator for 10 days. After this pre-incubation, 10.0 mg g⁻¹ of crushed straw was evenly mixed into the soil assigned to the straw treatments, equivalent to 1% (w/w). The soil moisture content of each bottle was then adjusted to 30% with distilled water and the bottles were sealed. The symmetric freeze–thaw samples were cooled to –10 °C for 12 h and then raised to 5 °C and thawed for 12 h, which constituted one freeze–thaw cycle (1 day). The asymmetric freeze–thaw samples were cooled to –10 °C for 60 h and then raised to 5 °C and thawed for 12 h, which constituted one freeze–thaw cycle (3 days). Samples not subjected to freeze–thaw treatment were incubated continuously at 5 °C. During the incubation the soil moisture content was adjusted regularly by the gravimetric method. Soil samples were collected on days 0, 9, 18, 27, 36 and 45 after the start of the experiment; after incubation each sample was divided for the different carbon fractions. Soil organic carbon (SOC) was determined on air-dried soil, whereas dissolved organic carbon (DOC), microbial biomass carbon (MBC) and the amino sugars used to calculate fungal and bacterial necromass carbon (FNC and BNC) were determined on fresh soil immediately after the bottle was taken out of the incubation.

Each of the six treatments was prepared in three independent bottles per soil and depth. Sampling was destructive: at every sampling time a separate set of bottles was taken out of the incubation and processed, so no bottle was used twice. The three replicate bottles of one treatment are therefore independent experimental units, and the six sampling times form independent groups rather than repeated measurements of the same unit. Sampling time was treated as a fixed factor together with soil type, soil depth, freeze–thaw regime and straw amendment in all statistical analyses. The complete design comprised two soils, two depths, three regimes, two straw treatments and three replicate bottles at each of six sampling times, which required 432 bottles in total. Because only one composite sample was obtained per site and depth, the three replicates are independent incubation units rather than independent field replicates, and the individual bottle is the experimental unit.

2.3. Soil Physicochemical Analysis

Among the many methods of pH determination, the electrode potentiometric method has been widely used in soil science research at home and abroad because of its ability to obtain results quickly and accurately [21], using the PHS-3C pH metre.

Soil physicochemical properties were determined following the methods described by Qu et al. [22]. Soil SSC was determined by the residue drying–mass method at a ratio of 5:1 (water to soil). The concentration of exchangeable sodium (cmol(Na⁺) kg⁻¹) was measured by flame photometry (Shimadzu optical double-beam atomic absorption spectrophotometer, Shanghai, China). Cation exchange capacity (CEC) (cmol kg⁻¹) was determined by the EDTA–ammonium acetate salt exchange method, and the exchangeable sodium percentage (ESP) was calculated from these values.

2.4. Amino Sugars

Amino sugars were determined according to the method described by Appuhn and Joergensen [23].

Soil samples (containing approximately 0.3 mg N) were heated with 6 M of 10 mL HCl solution at 105 °C for 8 h. After cooling, 100 mL of inositol solution (1 µg mL⁻¹) was added to filter the hydrolysate. After the hydrolysate was cooled to room temperature, 100 µL of

1 $\mu\text{g mL}^{-1}$ inositol solution (internal standard 1) was added, shaken well, and filtered with quantitative filter paper. The filtrate was evaporated and dried with a rotary evaporator, the residue was dissolved with distilled water, the pH value was adjusted to 6.6–6.8, and then centrifuged, the supernatant was evaporated with a rotary evaporator, and the residue was dissolved and centrifuged with methanol. The supernatant was transferred to a derivatization flask, dried with nitrogen (N_2) at 45 °C, then 1 mL of distilled water was added, freeze-dried and then derivatized by glyconitrile acetyl of amino sugars, the derivatives were determined by gas chromatography and a flame ionization detector (gas chromatograph-Agilent 6890, Agilent Technologies, Santa Clara, CA, USA), and amino sugars were calculated according to the internal standard method. The total amino sugar concentration was calculated as the sum of GluN, GalN and MurA. Some studies have shown that the ratio of MurA to GluN in bacteria is 1:2, so that the fungal glucosamine (GluNF, mg kg^{-1}) can be calculated [24].

2.5. Determination of Soil Organic Carbon Components

2.5.1. Soil Organic Carbon (SOC)

The SOC was tested using a total organic carbon analyzer (TOC-V, SSM-5000A) [25]. Specifically, 50.0 mg of air-dried soil samples were weighed into the sample boats, and the two sample boats were put into the TC and SIC reactors, respectively. The soil samples are directly incinerated at high temperatures in the closed TC reactor to produce CO_2 , and the soil samples in the SIC reactor are acidified by phosphorylation to produce CO_2 , and the CO_2 gas in the two reactors is passed through the non-dispersive infrared detector (NDIR), respectively, and the test results of the soil samples TC, SIC and SOC ($\text{SOC} = \text{TC} - \text{SIC}$) are calculated by the computer according to the comparison of the standard curve of the detection results. Set up 3 parallel samples per sample. In this study, the test results were converted to g kg^{-1} .

2.5.2. Soil Microbial Biomass Carbon (MBC)

Soil microbial biomass carbon (MBC) was determined by chloroform fumigation extraction [26].

Weigh the fresh soil equivalent to 10 g of drying soil weight and put it in a 50 mL polyethylene culture flask, put a small beaker with water at the bottom of the vacuum dryer, put 2 small beakers containing 30 mL NaOH solution and 1 small beaker containing 50 mL of non-ethyl alcohol chloroform and a polyethylene bottle containing soil samples into the dryer, seal and pump until chloroform boils for 2 min, and place it at 25 °C for 24 h in the dark. After the fumigation is completed, the air is pumped with a vacuum pump until there is no chloroform residue, 40 mL of 0.5 mol L^{-1} K_2SO_4 solution is added to the culture flask for 30 min, centrifuged, and measured by a TOC instrument after passing through a 0.45 μm filter membrane.

2.5.3. Dissolved Organic Carbon (DOC)

Determination of soil dissolved organic carbon (DOC) [26]: 8 g of fresh soil samples were weighed and placed in a 50 mL centrifuge tube, 40 mL of K_2SO_4 solution of 0.5 mol L^{-1} was added, and after shaking for 30 min, centrifugation was carried out at a speed of 15,000 r min^{-1} for 10 min, and then the content of DOC (mg kg^{-1}) was determined by the TOC instrument after passing through a 0.45 μm filter membrane.

2.5.4. Microbial Necromass Carbon (MNC)

Fungal necromass carbon (FNC) and bacterial necromass carbon (BNC) were calculated from the amino sugar contents as $\text{FNC} = (\text{GluN}/179.2 - 2 \times \text{MurA}/251.3) \times 179.2 \times 9$ and $\text{BNC} = \text{MurA} \times 45$, where GluN is glucosamine (mg kg^{-1}), MurA is muramic acid

(mg kg^{-1}), 179.2 and 251.3 are the molecular weights of GluN and MurA, respectively, 9 is the conversion factor from fungal-derived GluN to fungal necromass carbon and 45 is the conversion factor from muramic acid to bacterial necromass carbon. Total microbial necromass carbon (MNC) was calculated as the sum of FNC and BNC [27].

2.6. Statistical Analysis

All analyses were performed in R version 4.6.1 (R Core Team 2026) with RStudio version 2026.07.1, using the packages *car*, *agricolae*, *psych* and *factoextra*. Each incubation bottle was an independent experimental unit. Normality and homoscedasticity of residuals were checked with the Shapiro–Wilk and Levene tests, respectively, and variables were $\log_{10}$ -transformed where the assumptions were violated. The effects of freeze–thaw regime, straw amendment, soil type, soil layer and sampling time (all treated as fixed factors), together with their interactions, were evaluated using a five-factor analysis of variance. Type III sum of squares, implemented with the *Anova* function of the *car* package in R, was used so that each effect is tested after adjustment for all other terms in the model. Effect sizes are reported as partial η^2 , for which values of about 0.01, 0.06 and 0.14 represent small, medium and large effects. A separate factorial model containing freeze–thaw regime, straw amendment, soil type and soil depth was fitted to the day-45 data to characterize the end-point responses. Exact test statistics, degrees of freedom and partial η^2 values are given in Table 2 and Figure 4, and the treatment groupings in Table 3. Relative change rates were calculated as $(C_t - C_0)/C_0 \times 100\%$ and are expressed relative to the initial value of the same treatment so that all pools are reported on a common, dimensionless scale. Post hoc comparisons were made with Tukey’s HSD test ($\alpha = 0.05$) and are represented by the letter groupings in Table 3. significance is indicated throughout by asterisks (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Principal component analysis was performed on z-score-standardized relative changes using *prcomp* (*factoextra*). 4 Pearson correlation coefficients among the relative changes and the standing FNC/BNC ratio were calculated with *corr.test* (*psych*) and are presented in Table 4 and Figure 6. A full factorial model including sampling time was also fitted for each indicator; sampling time and its interaction with the freeze–thaw regime were significant for all six indicators, so sampling time was retained as a fixed factor and the day-45 end point is presented as the primary comparison.

Table 2. Factorial analysis of variance in the relative change (%) of each indicator on day 45.

Indicator	Regime	Regime $\times$ Straw	Soil type	Depth	R ²
DOC	30.5 ***	0.3 n.s.	6.8 *	8.8 **	0.867
MBC	78.7 ***	179.9 ***	8.6 **	6.3 *	0.974
MNC	66.0 ***	145.0 ***	0.0 n.s.	1.8 n.s.	0.954
FNC	58.1 ***	118.1 ***	2.4 n.s.	0.4 n.s.	0.932
BNC	21.7 ***	56.7 ***	5.0 *	16.9 ***	0.939
FNC/BNC	3.4 *	4.9 *	10.8 **	19.0 ***	0.788

Table 2 gives the F statistic of each term, with asterisks marking significance (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; n.s., not significant). The degrees of freedom are two for the freeze–thaw regime, one for soil type, one for soil depth and two for the regime $\times$ straw interaction, with 60 residual degrees of freedom.

Table 3. Treatment groupings for the relative change (%) of each indicator on day 45 (Tukey's HSD test, $p < 0.05$).

Indicator	CK	CK + S	SFT	SFT + S	ASFT	ASFT + S
DOC	c	b	b	b	a	a
MBC	c	b	d	c	e	a
MNC	b	c	c	c	d	a
FNC	b	c	c	c	d	a
BNC	b	b	c	b	d	a
FNC/BNC	a	b	a	b	a	b

Table 3 gives the treatment groupings obtained with Tukey's HSD test. Within a row, treatments that share a letter are not significantly different, and the letters are assigned from the highest to the lowest mean value, so that 'a' always denotes the highest-ranking group.

Table 4. Pearson correlation coefficients among the relative changes (%) in the five carbon pools on day 45 and the standing FNC/BNC ratio on day 45 ($n = 72$).

Indicator	FNC	MNC	BNC	MBC	DOC	FNC/BNC
FNC	1	0.97 ***	0.77 ***	0.78 ***	0.03 n.s.	0.07 n.s.
MNC	0.97 ***	1	0.91 ***	0.86 ***	0.06 n.s.	−0.06 n.s.
BNC	0.77 ***	0.91 ***	1	0.87 ***	0.09 n.s.	−0.23 n.s.
MBC	0.78 ***	0.86 ***	0.87 ***	1	0.11 n.s.	−0.12 n.s.
DOC	0.03 n.s.	0.06 n.s.	0.09 n.s.	0.11 n.s.	1	−0.33 **
FNC/BNC	0.07 n.s.	−0.06 n.s.	−0.23 n.s.	−0.12 n.s.	−0.33 **	1

Table 4 gives the correlation coefficients and their significances (** $p < 0.01$, *** $p < 0.001$; n.s., not significant).

3. Results

3.1. Responses of the Carbon Pools to Freeze–Thaw Regimes and Straw Amendment

Figure 2 shows the change in MNC after 45 days. The bars are means of three independent incubation bottles and the error bars are standard errors. Lowercase letters above the bars mark significant differences among the six treatments within a soil–depth combination (Tukey's HSD test, $p < 0.05$). MNC decreased most strongly under ASFT without straw and was highest under ASFT with straw in all four combinations. Averaged over the two soils and the two depths, MNC changed by +3.02% under the CK, −3.77% under the SFT and −10.29% under ASFT without straw, and by −3.03%, −3.23% and +8.88%, respectively, when straw was added. The soil–depth pairs differed mainly in the magnitude rather than in the direction of the response. The largest decrease occurred in the maize topsoil (−12.37% under ASFT) and the largest increase in the maize subsoil (+12.53% under ASFT with straw).

The interaction between the freeze–thaw regime and straw amendment is the most striking feature of the dataset and appears in Figure 2 as the opposite positions of the two ASFT bars in every panel. Straw amendment changed MNC by +19.17% under ASFT, by −6.05% under the CK and by +0.54% under the SFT. Resolved by soil and depth, the change ranged from +13.64% in the paddy topsoil to +23.09% in the maize subsoil. In the paddy soil the control treatment was statistically indistinguishable from ASFT with straw, whereas in the maize soil the control formed an intermediate group that was separated from both ASFT treatments. The same interaction was present in the two necromass fractions (Figure 3) and in the microbial biomass, and it is quantified in Tables 2 and 3 and in Figure 4.

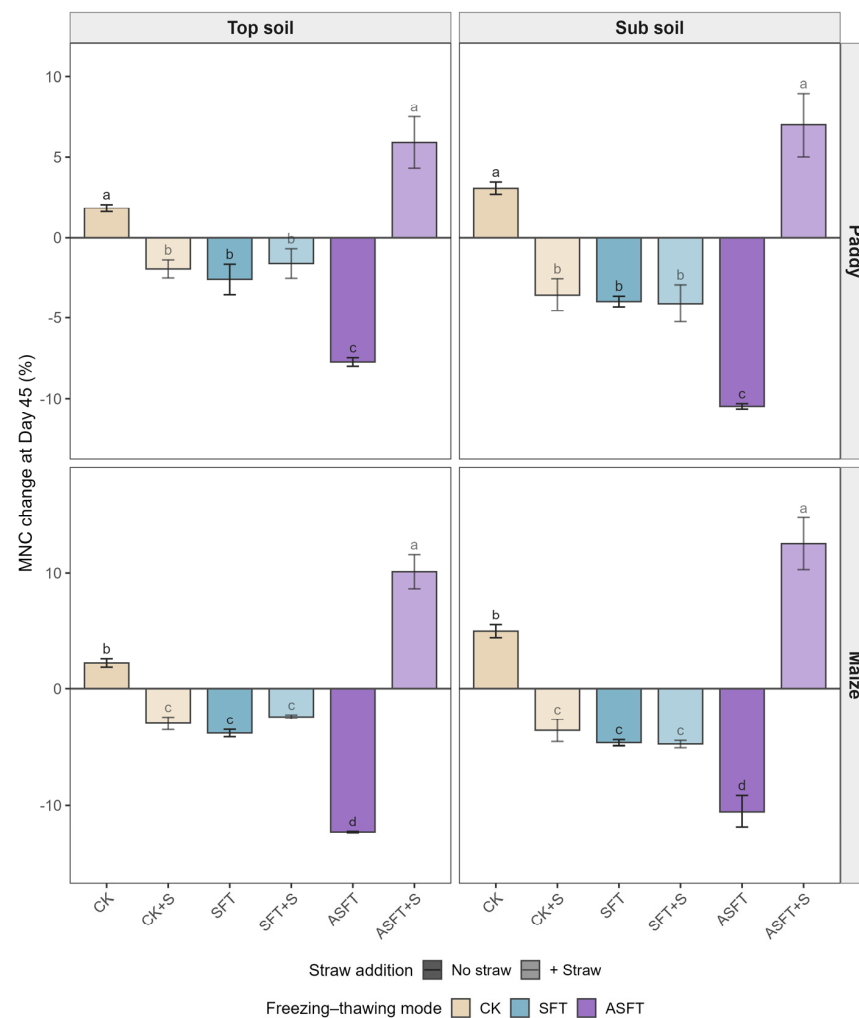


Figure 2. Relative change (%) in microbial necromass carbon (MNC) after 45 days of incubation under the six treatments, shown separately for the topsoil (0–20 cm) and the subsoil (20–40 cm) of the paddy and maize soils. Bars are means of three independent incubation bottles and error bars are standard errors. Lowercase letters above the bars indicate significant differences among the six treatments within a soil–depth combination (Tukey’s HSD test, $p < 0.05$). CK, constant 5 °C; SFT, symmetric freeze–thaw; ASFT, asymmetric freeze–thaw; and +S, with straw amendment.

These results support H1 and H3. The asymmetric regime was associated with the strongest depletion of MNC and BNC, as expected for the regime with the longest freezing phase, and straw amendment reduced that depletion under the same regime. The FNC/BNC ratio rose above its initial value in all three treatments without straw, which is consistent with H2, although the change in the ratio was much smaller than the changes in the pools themselves.

The freeze–thaw regime affected all six indicators, with $F = 30.5, 78.7, 66.0, 58.1, 21.7$ and 3.4 for DOC, MBC, MNC, FNC, BNC and the FNC/BNC ratio, respectively. The regime $\times$ straw interaction was significant for MBC, MNC, FNC, BNC and the FNC/BNC ratio ($F = 179.9, 145.0, 118.1, 56.7$ and 4.9) but not for DOC ($F = 0.3$, n.s.), so the interaction was specific to the biomass and necromass pools. Straw amendment also had a highly significant main effect on every indicator except the FNC/BNC ratio, and its magnitude is reflected in the treatment groupings of Table 3 and in the paired bars of Figures 2 and 4. Soil type and depth contributed little to MNC and FNC ($F \leq 2.4$, n.s.), were significant for BNC ($F = 5.0$ and 16.9) and dominated the FNC/BNC ratio ($F = 10.8$ and 19.0). The models explained between 78.8% (FNC/BNC ratio) and 97.4% (MBC) of the variance in the day-45

responses, so the four experimental factors and their interaction account for most of the variation in this dataset. The partial η^2 values of the same terms are shown in Figure 4.

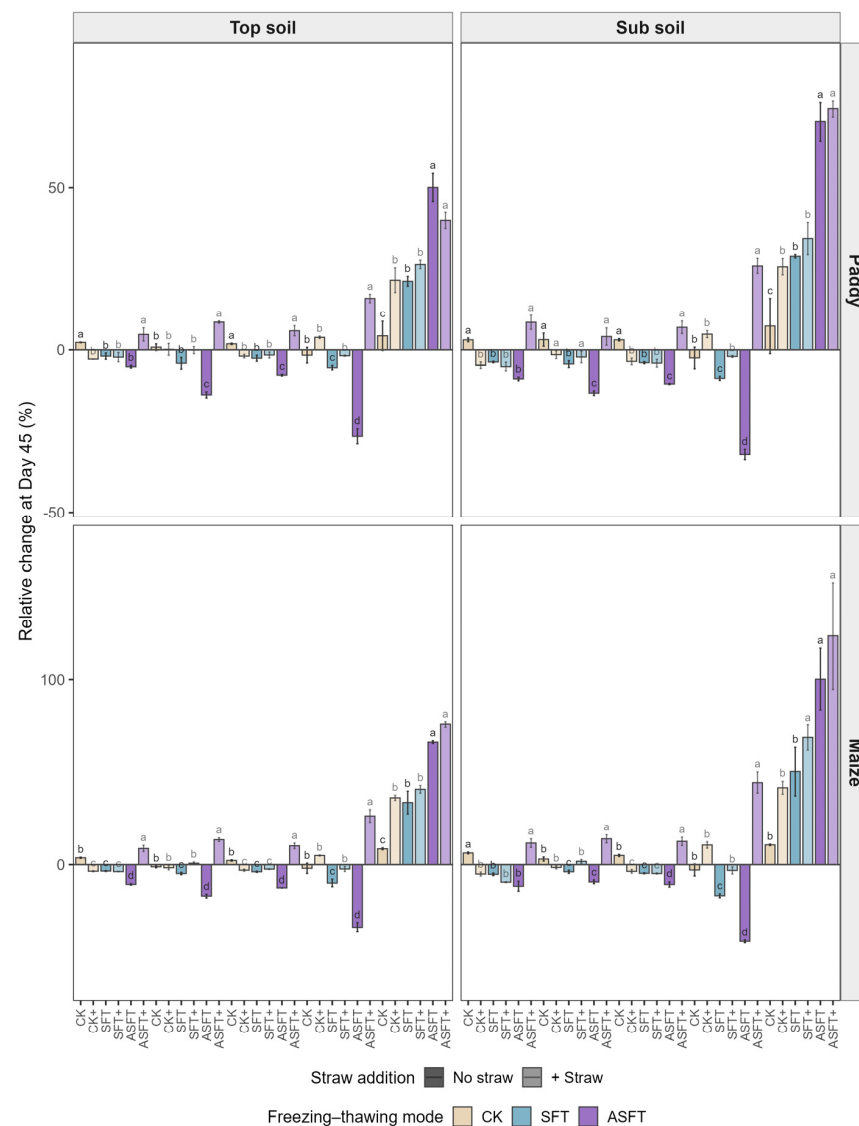


Figure 3. Relative change (%) on day 45 in fungal necromass carbon (FNC), bacterial necromass carbon (BNC), microbial necromass carbon (MNC), microbial biomass carbon (MBC) and dissolved organic carbon (DOC), shown separately for the topsoil (0–20 cm) and the subsoil (20–40 cm) of the paddy and maize soils. Bars are means of three independent incubation bottles and error bars are standard errors. Lowercase letters above the bars indicate significant differences among the six treatments within a soil–depth combination (Tukey's HSD test, $p < 0.05$).

The groupings separate the treatments in the same way for BNC, MNC and FNC. In each of these three indicators ASFT with straw ranks the highest (group a), the three intermediate treatments form the overlapping groups b and c, and ASFT without straw ranks the lowest (group d), so the two asymmetric treatments differ significantly from each other and from all other treatments. MBC follows the same order but covers a wider range, from group e for ASFT without straw to group a for ASFT with straw. DOC behaves differently. It reaches its highest values under both asymmetric treatments (group a) and its lowest value under the CK (group c), and it does not separate the with-straw from the without-straw treatment within a regime. For the FNC/BNC ratio the three treatments without straw form group a and the three treatments with straw form group b, so straw amendment rather than the freeze–thaw regime determines the position of a treatment in this ratio.

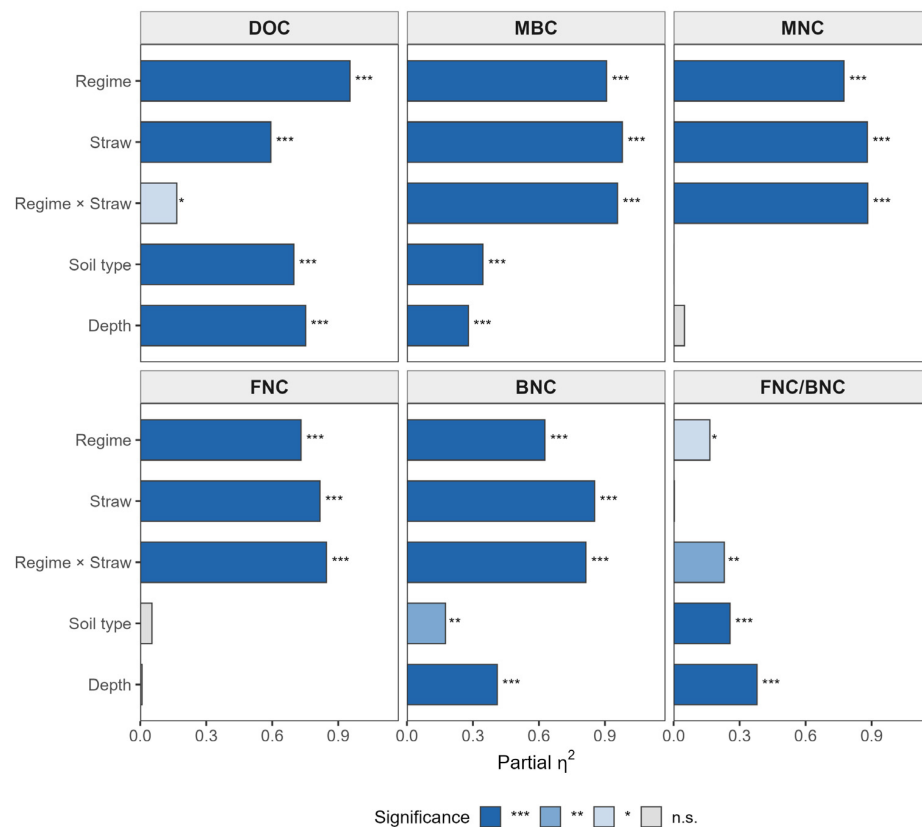


Figure 4. Effect sizes (partial η^2) of each experimental factor for the relative change in every indicator on day 45 in the four-factor model. Each bar gives the partial η^2 of one term (freeze–thaw regime, straw amendment, soil type, soil depth, or the regime $\times$ straw interaction), with bar shading and asterisks marking significance (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; n.s., not significant).

Figure 3 uses the same conventions as Figure 2 and shows how the five carbon pools responded together in each soil–depth combination. The necromass pools (FNC, BNC and MNC) and MBC all decreased under ASFT without straw and increased under ASFT with straw, so the two asymmetric treatments occupied the extremes of the axis. Averaged over soils and depths, MBC changed from -33.52% to $+28.17\%$, MNC from -10.29% to $+8.88\%$, FNC from -9.01% to $+8.41\%$ and BNC from -13.27% to $+10.01\%$. DOC increased in every treatment, by 7.71% under the CK, 33.49% under the SFT and 71.6% under ASFT without straw, and by 31.27% , 42.49% and 78.21% when straw was added, so its ranking of treatments was determined by the freeze–thaw regime rather than by straw amendment.

The four panels also reveal consistent differences among the pools. MBC was proportionally the most responsive of the five. It fell by 33.52% under ASFT without straw, which is three times the decrease in MNC (-10.29%) and more than twice that in BNC (-13.27%), and this indicates that the living biomass reacted more strongly than the necromass it produces. BNC was the most responsive of the necromass pools in every panel, and the difference between BNC and FNC was largest under the asymmetric regime, where the ratio of their relative changes reached ≈ 1.5 . DOC increased more than any other pool in every treatment, most strongly in the maize subsoil ($+125.9\%$ under ASFT with straw) and least in the paddy topsoil ($+17.8\%$ under CK), which parallels the larger pool size and the stronger freeze–thaw contrast in the maize soil.

Figure 4 gives the partial η^2 of each term, with bar shading and asterisks marking significance (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Partial η^2 expresses the proportion of the residual variance accounted for by each term and is independent of the units in which the pools are expressed. For MNC, FNC, BNC and MBC the straw main effect and the regime $\times$ straw

interaction were the two largest terms (partial $\eta^2 = 0.70$ – 0.88), and both exceeded the soil type and depth terms in every case. For DOC the regime main effect dominated (partial $\eta^2 = 0.56$) and the interaction was negligible ($\eta^2 = 0.01$). The FNC/BNC ratio showed the reverse pattern. Depth and soil type were its strongest terms (partial $\eta^2 = 0.28$ and 0.18) and the straw main effect was effectively zero ($\eta^2 = 0.001$), so only the interaction remained significant. Straw amendment therefore acted on the fungal and bacterial pools in a coordinated manner, changing their absolute amounts without shifting their ratio.

3.2. Multivariate Structure and Correlations Among the Carbon-Pool Changes

Figure 5 shows the ordination of the 72 day-45 samples. Symbols represent individual incubation bottles, coloured by freeze–thaw regime and shaped according to straw addition, and the arrows show the loadings of the five relative changes and of the standing FNC/BNC ratio. The first two principal components explained 60.0% and 22.4% of the variance, so together they summarized 82.4% of the variation in the day-45 data. PC1 was loaded almost equally by the changes in MNC (0.52), BNC (0.50), MBC (0.49) and FNC (0.49) and therefore represents the overall direction and magnitude of change in the biomass and necromass pools. The changes in DOC (0.07) and the FNC/BNC ratio (-0.07) contributed almost nothing to it. PC2 contrasted the standing FNC/BNC ratio (0.71) with the change in DOC (-0.67), so it separates samples that combine a high fungal-to-bacterial ratio with little dissolved carbon accumulation from those with the opposite combination.

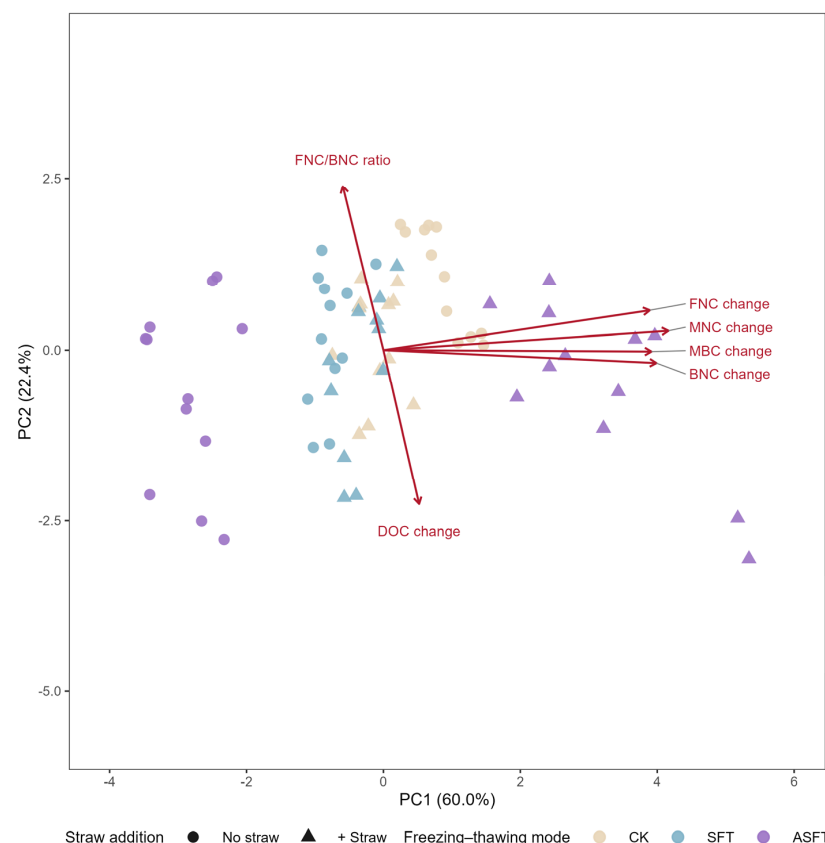


Figure 5. Principal component analysis of the relative changes in the five carbon pools and of the standing FNC/BNC ratio after 45 days ($n = 72$). Symbols are individual incubation bottles, coloured by freeze–thaw regime and shaped according to straw amendment, and arrows give the loadings of the six variables.

The ASFT treatment amended with straw had the highest PC1 scores and ASFT without straw the lowest, so the first axis orders the treatments from the strongest suppression

to the strongest accumulation of biomass and necromass carbon. Along PC2, the ASFT samples amended with straw extended towards negative values, that is towards a high FNC/BNC ratio and a small increase in DOC, whereas the CK samples remained close to the origin. The ordination is interpreted in terms of the relative contributions of fungal- and bacterial-derived necromass rather than in terms of the microbial community structure, which was not measured in this study.

Figure 6 shows the correlations among the five relative changes and the standing FNC/BNC ratio, and the numerical values and the significance of each coefficient are given in Table 4. The four biomass- and necromass-related variables (MBC, BNC, MNC and FNC) were internally and strongly correlated, with coefficients ranging from 0.77 to 0.97 (all $p < 0.001$), so microbial biomass carbon and the two necromass fractions changed in a coordinated way. The change in MBC was closely coupled to the change in BNC ($r = 0.87$, $p < 0.001$), which indicates synchronized dynamics between bacterial biomass and bacterial necromass. DOC was uncorrelated with every other variable ($|r| \leq 0.11$, all n.s.) and therefore formed a separate group. The standing FNC/BNC ratio was negatively correlated with the change in DOC ($r = -0.33$, $p < 0.01$) and weakly so with BNC (-0.23 , n.s.) and MBC (-0.12 , n.s.), and it was unrelated to the change in MNC (-0.06 , n.s.) and FNC (0.07 , n.s.).

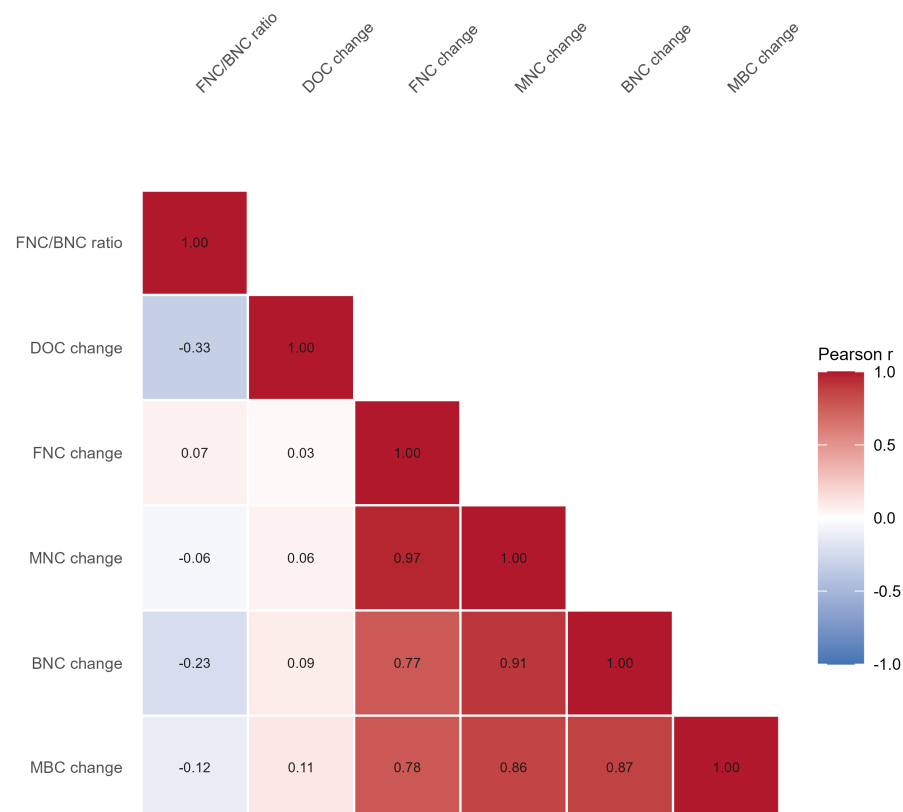


Figure 6. Pearson correlations among the relative changes in the five carbon pools on day 45 and the standing FNC/BNC ratio ($n = 72$). Cells give the correlation coefficient; the numerical values and their significance are given in Table 4.

Because MNC is defined as the sum of FNC and BNC, the coefficients involving MNC and its two components are partly constrained by that definition and are interpreted as compositional relationships rather than as independent evidence of causation. The coefficients that are not constrained by the definition are those between FNC and BNC ($r = 0.77$, $p < 0.001$), between MBC and BNC ($r = 0.87$, $p < 0.001$), between MBC and FNC ($r = 0.78$, $p < 0.001$), and between the standing FNC/BNC ratio and DOC ($r = -0.33$, $p < 0.01$). The

table is consistent with the structure seen in Figure 6. The strongest relationships are those among MBC, BNC, MNC and FNC, whereas DOC occupies a separate position and none of its coefficients is significant, its highest correlation with any of the pool changes being 0.11. The standing FNC/BNC ratio was significantly correlated only with the change in DOC, which indicates that a higher fungal-to-bacterial necromass ratio was associated with a smaller accumulation of dissolved carbon. Taken together, Figure 6 and Table 4 show that the response of the soil carbon pools to freeze–thaw and straw amendment was organized mainly along a biomass–necromass axis, while the dissolved fraction responded independently.

4. Discussion

4.1. Responses of the Carbon Pools to Contrasting Freeze–Thaw Regimes

The asymmetric regime was the most divergent treatment for every carbon pool. Relative to the initial value it reduced BNC by 13.27%, MNC by 10.29%, FNC by 9.01% and MBC by 33.52%, while increasing DOC by 71.6%. Straw amendment reversed the sign of the response of all three necromass pools under that regime, moving MNC from −10.29% to +8.88%, FNC from −9.01% to +8.41% and BNC from −13.27% to +10.01%, changes of between +17.42% and +23.28%. The straw main effect and the regime × straw interaction were the two largest terms in the models for MNC, FNC, BNC and MBC (partial $\eta^2 = 0.70$ – 0.88) and both exceeded soil type and depth in every case. The four biomass- and necromass-related indicators changed in a coordinated way ($r = 0.77$ – 0.97 , all $p < 0.001$), whereas DOC was uncorrelated with all of them ($|r| \leq 0.11$, all n.s.). Soil type and depth dominated the FNC/BNC ratio (partial $\eta^2 = 0.18$ and 0.28) and the straw main effect on that ratio was effectively zero, so the composition of the necromass pool reflected the intrinsic properties of the soil more closely than the incubation treatment.

The frequency and duration of freeze–thaw cycles affect the accumulation and composition of microbial necromass carbon [28,29]. In this study the symmetric (12 h/12 h) and asymmetric (12 h/60 h) regimes differed markedly in their association with FNC and BNC. Averaged over soil and depth, the asymmetric regime without straw reduced MNC by 10.29% and BNC by 13.27%, whereas the symmetric regime produced much smaller changes (−3.77% and −4.29%). The difference between the two regimes was significant for both pools (Tables 2 and 3), which indicates that the temporal structure of the freeze–thaw cycles, rather than the occurrence of freezing as such, is the factor most closely associated with microbially derived carbon cycling in these soils.

Under symmetric freeze–thaw conditions, FNC and BNC in the no-straw treatment both declined slightly and fluctuated, which may be related to the repeated rupture of microbial cells caused by frequent freeze–thaw cycles and to the continuous release and decomposition of necromass carbon [30]. High-frequency temperature fluctuations may accelerate the breakdown of soil aggregates, exposing physically protected microbial necromass to decomposers and promoting its mineralization. At the end of the incubation the FNC/BNC ratio in the no-straw treatments was above its initial value under all three regimes (+2.41% under CK, +0.75% under SFT and +5.08% under ASFT), which is consistent with a proportionally greater loss in BNC than in FNC; this may be related to the greater physical resistance of fungal hyphae and to the recalcitrance of cell wall components such as chitin [31–33]. The largest increase in the ratio occurred under the asymmetric regime, where BNC fell by 13.27% but FNC by only 9.01%. The long freezing phase of the asymmetric regime (60 h) may be associated with more severe physiological drought and osmotic stress and therefore with greater freezing damage to bacterial communities. Intracellular substances released after cell death may be rapidly decomposed and utilized during the brief thawing period, which would make it difficult for them to be transformed

effectively into stable necromass carbon [34]. These observations are consistent with reports of a shift towards fungal dominance in microbial communities of seasonal freeze–thaw regions [35], where long-term freezing environments favour more stress-tolerant fungal communities while bacterial biomass and its necromass contribution decline relatively. It should be emphasized, however, that fungal and bacterial dominance is inferred here from necromass biomarkers and not from a direct measurement of community composition.

A note of caution is required when comparing the two freeze–thaw regimes. The symmetric and asymmetric treatments differ simultaneously in the duration of each freezing phase, the duration of each thawing phase, the length of the cycle, the number of cycles completed within the 45-day incubation, and the cumulative freezing and thawing exposure. Because these components were not manipulated independently, the present design cannot attribute the observed differences to any single one of them, and the treatments are therefore described as two contrasting freeze–thaw regimes rather than as a separation of duration and frequency. Disentangling the two effects would require a crossed factorial design in which freezing duration and cycle frequency are varied independently.

The two active carbon pools also differed between the regimes, and not in the same direction. Under symmetric freeze–thaw, MBC decreased by 10.14% and DOC increased by 33.49%; under asymmetric freeze–thaw, MBC decreased by 33.52% and DOC increased by 71.6%. The asymmetric regime therefore showed both the strongest loss in living microbial biomass and the largest accumulation of dissolved carbon. This coincident pattern is compatible with microbial biomass turnover as one possible source of DOC, but the present dataset cannot distinguish this source from other potential contributions. MBC fell by 33.52% while MNC fell by only 10.29%, so the biomass pool was proportionally more affected than the necromass pool it generates. This is consistent with a lag between the loss in living biomass and the response of the necromass fraction.

DOC is a mixed pool. It can originate from microbial cell lysis, straw decomposition, mineralization of native organic matter, desorption from mineral surfaces, disruption of aggregates and release of root- and microbe-derived exudates, and the present dataset does not allow these sources to be separated. The absence of a significant correlation between DOC change and the necromass-related indicators (Table 4, $|r| \leq 0.11$) therefore indicates only that DOC changes were not linearly associated with necromass accumulation, and it does not identify the dominant source. Partitioning the sources would require ^{13}C -labelled straw tracing or molecular characterization of DOC such as FT-ICR-MS or EEM-PARAFAC.

4.2. Straw Amendment and the Balance Between Fungal and Bacterial Necromass

Straw addition, as an input of exogenous organic carbon, was associated with marked changes in the accumulation dynamics of FNC and BNC under the different freeze–thaw regimes [36]. Averaged over soil and depth, the largest single effect in the dataset was the increase in MBC under the asymmetric regime, which rose from -33.52% without straw to $+28.17\%$ with straw, a difference of $+61.7\%$. The corresponding differences were $+23.28\%$ for BNC and $+19.17\%$ for MNC. The same amendment applied at constant temperature produced the opposite response in the necromass pools (MNC, -6.05%) and, under the symmetric regime, almost no change in MNC ($+0.54\%$).

Under constant-temperature incubation at $5\text{ }^{\circ}\text{C}$, FNC and BNC in the straw-amended treatment both declined relative to the no-straw treatment (MNC from $+3.02\%$ to -3.03% , FNC from $+3.82\%$ to -4.05% and BNC from $+1.44\%$ to -1.13%), which indicates that exogenous organic carbon input did not promote MNC accumulation under stable temperature conditions. On the contrary, the addition of fresh straw may have triggered a “cometabolic effect” or “priming effect” [37,38], accelerating the decomposition of native soil organic matter and resulting in net losses of necromass carbon. The FNC/BNC ratio

in the straw-amended treatment was lower than its initial value and lower than that in the no-straw treatment (-2.93% versus $+2.41\%$), which is consistent with a proportionally greater reduction in FNC than of BNC. This may be related to the selective utilization of recalcitrant straw components such as lignin, since fungi have metabolic advantages in decomposing complex organic matter [39].

Under symmetric freeze–thaw conditions, straw addition had no significant effect on MNC or FNC (both treatments fall in group c in Table 3) but was associated with higher BNC than in the no-straw treatment, which moved from group c to group b and changed by $+4.33\%$. Exogenous organic carbon input therefore alleviated, to some extent, the negative association between freeze–thaw and BNC accumulation. The readily available carbon sources released during straw decomposition may have supported the rapid recovery and growth of bacterial communities, enabling them to rebuild biomass and form necromass carbon more quickly during freeze–thaw intervals [40]. This compensatory effect was limited for FNC, which suggests that fungal necromass carbon accumulation was more closely associated with the physical disturbance caused by freeze–thaw than with substrate availability.

Under asymmetric freeze–thaw conditions, the effect of straw addition was the most pronounced feature of the dataset (Figures 2 and 5). FNC in the straw-amended treatment exceeded its initial value ($+8.41\%$), and BNC was also higher than its initial value ($+10.01\%$), whereas both FNC and BNC in the no-straw treatment fell below their initial values (-9.01% and -13.27%). This contrast indicates that exogenous organic carbon input was associated with a reversal of the negative response of MNC accumulation under the asymmetric regime, with the most prominent association for BNC ($+23.28\%$). Substrate limitation during the long freezing phase of the asymmetric regime may be a key factor restricting necromass carbon accumulation, and straw addition supplemented carbon sources and energy, supporting microbial growth, metabolism and necromass formation during thawing periods. Because the straw effect on MNC under ASFT ($+19.17\%$) was far larger than under CK (-6.05%) or SFT ($+0.54\%$), the benefit of the straw was specific to the asymmetric regime rather than a general property of the amendment.

The FNC/BNC ratio in the straw-amended treatments was below its initial value under all three regimes (-2.93% under CK, -5.08% under SFT and -1.34% under ASFT), which differs from the pattern observed for the treatments without straw. Taken together with the non-significant straw main effect on the relative change in the ratio ($F = 0.0$, n.s. in Table 2) and with the fact that the two straw groups form the same two groups for every regime in Table 3, this indicates that straw amendment did not shift the composition of the necromass pool but rather that the two components changed in parallel [41].

Three indicators deserve particular emphasis. BNC was the most responsive of the six. It was the only necromass pool for which soil depth was significant ($F = 16.9$, Table 2), it showed the largest absolute change in the necromass pools under the regime $\times$ straw interaction ($+23.28\%$), and its grouping structure in Table 3 is the clearest of all six indicators, with the six treatments resolved into four distinct groups. MBC responded more strongly than any other indicator to straw amendment ($+61.7\%$ under ASFT), which indicates that the living biomass reacted faster and more strongly than the necromass pools and that part of the necromass response is likely to develop after the period studied here. The FNC/BNC ratio behaved differently, because its variation was governed mainly by soil type and depth (partial $\eta^2 = 0.18$ and 0.28 , Figure 4) rather than by the incubation treatments, so the composition of the necromass pool reflects the intrinsic properties of the two soils and the two depths more closely than the treatments imposed during the incubation.

It is important to distinguish three conceptually different quantities when interpreting these data. Our measurements directly quantify the accumulation of microbial necromass

carbon (FNC, BNC and MNC). A potential contribution of that necromass to soil organic carbon stabilization can only be inferred, because stabilization depends on mineral association, occlusion within aggregates, accessibility to decomposers and the prevailing environmental conditions, none of which were measured here. Actual long-term carbon stabilization cannot be assessed from a 45-day laboratory incubation. The present interpretation is therefore restricted to the measured accumulation of necromass, and the other two quantities are treated as hypotheses to be tested in future work. In particular, the accumulation of microbial necromass is not equated with stable soil organic carbon.

4.3. Limitations and Future Directions

Several limitations of this study should be noted. The incubation lasted 45 days and was conducted without plants, at constant moisture (30% water-holding capacity) and without the natural temperature fluctuations of the field. The results therefore describe the behaviour of the incubated soil material rather than of the field soil, and the magnitudes reported here should not be transferred directly to field conditions. Sampling was destructive and followed a parallel-sample design, so the six sampling times are independent groups rather than repeated observations of the same unit; the temporal trend is therefore inferred from between-group comparisons, and within-bottle trajectories cannot be followed. Only one composite soil sample was obtained per site and depth, so the three replicates are independent incubation units rather than independent field replicates, and the comparison between the paddy and maize soils is confounded with site and is not replicated at the field level, even though soil type was a significant term for several indicators (Table 2). Microbial community composition was not measured, so the interpretation in terms of fungal and bacterial contributions is based on necromass biomarkers (FNC and BNC) and should be regarded as an inference; testing it would require 16S rRNA/ITS amplicon sequencing, qPCR or PLFA analysis. The chemical composition of the straw (cellulose, hemicellulose, lignin, ash, total C and N) was also not characterized in detail, because the straw is used here as a uniform exogenous carbon input applied at a single rate rather than as a study object in its own right, and the possibility that different straw types would produce different responses therefore remains untested. The two freeze–thaw regimes differ simultaneously in the duration of the freezing phase, the duration of the thawing phase, the length of the cycle and the number of cycles completed within the incubation, so the contribution of each of these components cannot be separated from the others.

Future research should separate the components of freeze–thaw regimes using a crossed factorial design in which freezing duration and cycle frequency are varied independently, so that the confounding identified above can be removed. Microbial community composition should be characterized directly by 16S rRNA/ITS amplicon sequencing, qPCR and PLFA analysis, so that the interpretation in terms of fungal and bacterial contributions can be tested rather than inferred. ¹³C-labelled straw tracing, combined with a full characterization of straw composition, would make it possible to partition the sources of dissolved organic carbon and to quantify the formation of necromass from the added substrate. Laboratory findings should also be extended to the field through multi-year monitoring of seasonal freeze–thaw sequences, combined with measurements of mineral-associated organic carbon and aggregate fractions, in order to determine whether necromass accumulation translates into stabilized soil organic carbon.

The three hypotheses can now be assessed together. H1 is supported, because the asymmetric regime reduced MNC and BNC more strongly than the symmetric regime or the constant-temperature control in every soil–depth combination. H2 is partly supported: the standing FNC/BNC ratio rose above its initial value in the treatments without straw, as expected, but the effect of the freeze–thaw regime on that ratio was small compared with

the effect of soil type and depth. H3 is supported, because straw amendment reversed the depletion of all three necromass pools under the asymmetric regime, whereas at constant temperature it was associated with the opposite response.

5. Conclusions

(1) Over the 45-day incubation the two freeze–thaw regimes were associated with different responses in all six indicators. The asymmetric regime differed most strongly from the constant-temperature control: after 45 days, BNC had decreased by 13.27%, MNC by 10.29%, FNC by 9.01% and MBC by 33.52%, while DOC had increased by 71.6%. Because the two regimes differ simultaneously in freezing duration, cycle length and cycle frequency, these differences cannot be attributed to the freezing duration rather than to frequency and separating the two components would require a crossed factorial design.

(2) Straw amendment reversed the response of the necromass pools under the asymmetric regime: MNC moved from -10.29% to $+8.88\%$, FNC from -9.01% to $+8.41\%$ and BNC from -13.27% to $+10.01\%$, changes of between $+17.42\%$ and $+23.28\%$. Under constant temperature the same amendment reduced MNC from $+3.02\%$ to -3.03% , and under the symmetric regime it had no significant effect on MNC or FNC. The straw main effect and the regime $\times$ straw interaction were the two largest terms in the models for MNC, FNC, BNC and MBC (partial $\eta^2 = 0.70\text{--}0.88$), exceeding soil type and depth in every case. These results are consistent with substrate supply, rather than the freeze–thaw regime alone, being an important constraint on necromass accumulation in these soils, and they identify straw amendment as a promising management option for the asymmetric freeze–thaw conditions that prevail during winter in this region.

(3) The measured carbon pools fell into two weakly coupled groups. The biomass and necromass indicators (MBC, BNC, MNC and FNC) changed in a coordinated way ($r = 0.77\text{--}0.97$, all $p < 0.001$, Table 4), whereas DOC was uncorrelated with all of them ($|r| \leq 0.11$, all n.s.), so the accumulation of dissolved carbon under the asymmetric regime was not linearly related to necromass formation. Among the six indicators, BNC responded most strongly to the treatments and was the only necromass pool for which soil depth was significant, while the FNC/BNC ratio depended mainly on soil type and depth (partial $\eta^2 = 0.18$ and 0.28) and was unaffected by straw amendment as a main effect. BNC and the FNC/BNC ratio are therefore recommended as complementary indicators, the first for the amount of bacterial necromass and the second for the composition of the necromass pool, in further work on freeze–thaw stress and on organic carbon management in saline–alkali soils. Taken together, these results support H1 and H3, whereas H2 received only partial support, because the composition of the necromass pool was governed mainly by soil type and depth rather than by the incubation treatments.

These conclusions are based on a 45-day laboratory incubation of two saline–alkali soils and on necromass biomarkers rather than on direct measurements of microbial community composition, mineral association or long-term carbon stabilization, and the magnitudes reported here describe the incubated soil material rather than the field soil. Future work should separate the components of freeze–thaw regimes in a crossed factorial design, characterize the microbial community directly by molecular methods, trace the fate of added straw carbon using ^{13}C labelling, and extend the measurements to multi-year field monitoring together with mineral-associated organic carbon and aggregate fractionation, so that it can be determined whether the changes observed here translate into stabilized soil organic carbon.

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Abbreviations

The following abbreviations are used in this manuscript:

SOC	Soil Organic Carbon
DOC	Dissolved Organic Carbon
MBC	Microbial Biomass Carbon
MNC	Microbial Necromass Carbon
FNC	Fungal Necromass Carbon
BNC	Bacterial Necromass Carbon
ESP	Exchangeable Sodium Percentage
SSC	Soil Salt Content
CEC	Cation Exchange Capacity
ASFT	Asymmetric freeze–thaw
SFT	Symmetric freeze–thaw
FNC/BNC	Fungal Necromass Carbon/Bacterial Necromass Carbon

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