Elsevier Editorial System(tm) for Soil Biology and Biochemistry

Manuscript Draft

Manuscript Number: SBB16843R2

Title: Organic amendments change soil organic C structure and microbial community but not total organic matter on sub-decadal scales

Article Type: Research Paper

Keywords: Corn stover retention; Rye cover crop; FT-ICR-MS; Phospholipid fatty acid; 16S rRNA gene; Bacterial community

Corresponding Author: Dr. James Michael Tiedje,

Corresponding Author's Institution: Michigan State University

First Author: Jiangbing Xu

Order of Authors: Jiangbing Xu; Sarah S Roley; Malak M Tfaily; Rosalie K Chu; James Michael Tiedje

Manuscript Region of Origin: USA

Highlights

- Soil carbon was characterized by ion cyclotron resonance mass spectrometry
- Corn stover and rye cover crop changed composition of extractable soil organic C.
- Corn stover retention enhanced lignin-like compounds
- Cover crop enriched more condensed hydrocarbons
- Soil bacterial community composition corresponded to certain SOM compositions

Title page

1. Manuscript title:

Organic amendments change soil organic C structure and microbial community but not total organic matter on sub-decadal scales

2. Author names and affiliations:

Jiangbing Xu [a, b], Sarah S. Roley [c,1], Malak M. Tfaily [d,2], Rosalie K. Chu [d], James M. Tiedje [b]

[a] Institute of Ecology, School of Applied Meteorology, Nanjing University of Information Science and Technology, Nanjing 210044, China

[b] Center for Microbial Ecology, Michigan State University, MI 48824, United States

[c] Great Lakes Bioenergy Research Center and W.K. Kellogg Biological Station, Michigan State University, Hickory Corners, MI 49060, USA

[d] Environmental Molecular Sciences Laboratory, Earth and Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA 99354, USA

3. Corresponding author:

Dr. James M Tiedje

E-mail: tiedjej@msu.edu

---

[1] Present address: School of the Environment, Washington State University, Richland, WA 99354, USA

[2] Present address: Department of Environmental Science, University of Arizona, Tucson, AZ 85721, USA

Abstract

Organic C has many benefits for soil, but it is depleted by tillage and crop harvest, and especially so for biofuel crops. Accordingly, strategies such as partially retaining stover or planting a cover crop can help ameliorate the negative effect of C removal. We used a long-term field experiment to study the impacts of stover retention and planting a cover crop on soil organic matter (SOM), its extractable components, and the soil microbial community. SOM chemical composition characterization was determined by electrospray ionization (ESI) coupled with Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) in sequential water, methanol (MeOH), and chloroform ($CHCl_3$) extracts. The characteristics of the soil bacterial community were measured by phospholipid fatty acid (PLFA), real-time quantitative PCR, and 16S rRNA gene sequence. The variations in total SOM content, total microbial biomass, and bacterial population were slight among treatments, but SOM chemical compounds, arbuscular mycorrhizal fungi (AMF) biomass, and bacterial structure changed significantly, and especially so in the coupled application of stover retention and cover crop. Specifically, stover retention enriched more lignin-like compounds in soil, whereas cover crop enriched more condensed hydrocarbons, and had more compounds with an aromaticity index (AI) >0.5. The bacterial community was not altered by the cover crop, but the corn stover retention increased the relative abundances of Myxococcales (Deltaproteobacteria) and decreased that of Actinobacteria. Redundancy analysis (RDA) further revealed that the bacterial

community in the stover treatments had a significant positive association with $CHCl_3$-extracted chemical classes, i.e. unsaturated hydrocarbons and lipids, with the coupled application (stover and cover crop), and lignin and proteins with the corn stover only treatment. Taken together, our study shows how different C addition practices influence the molecular composition of SOM and the structure of soil microbial communities.

Keywords: Corn stover retention; Rye cover crop; FT-ICR-MS; Phospholipid fatty acid; 16S rRNA gene; Bacterial community

1. Introduction

Corn (*Zea mays* L.) residue has served as a feedstock for cellulosic ethanol production in the United States because of its high cellulose content and easy availability (Blanco-Canqui and Lal, 2009; Stewart et al., 2018), but this results in removal of corn stover and hence reduced carbon (C) sequestration in soil. Studies have suggested that the threshold levels of residue removal must be assessed for principal soil types based on the needs to maintain or enhance soil productivity. Besides, agronomic strategies such as cover crops, diverse crop rotations, and manure application, are employed to compensate the C loss by stover removal.

Planting a cover crop between periods of regular crop production increases soil organic matter (SOM) by adding biomass C and improving soil aggregation to protect SOM (Ruis and Blanco-Canqui, 2017; Stetson et al., 2012). In north central USA, winter rye (*Secale cereale*) is suggested as a preferred cover crop species because it is cold tolerant, vigorous, and cost-effective (Martinez-Feria et al., 2016). In addition, winter rye meshes well with a corn crop in the winter niche (Wilke and Snapp, 2008). C inputs from rye could replace some of the C removed in stover, although its capacity to directly increase SOM varies with agricultural management and soil type (Austin et al., 2017; Villamil et al., 2006; Cates and Jackson, 2019). Given the advantages of the rye cover crop with corn and its potential substitution for stover, we sought to evaluate these treatments at a more detailed biochemical and microbiological level.

SOM responds to organic amendments by shifting its quality and quantity, but defining its molecular composition and functional properties is still a challenge, in part because of the vast array of compounds. SOM exists on a humification continuum; operationally defined extractions have been extensively used to fractionate SOM into different classes to reduce heterogeneity (Ohno et al., 2010). Diverse technologies have been applied to elucidate the structure and function of the derived fractions after extraction, from chemical compositions of SOM (i.e. humin, humic acid, and fulvic acid) (Stevenson, 1994), to the fluorescence spectroscopy based on the multiple peaks and their specific location (Sanchez et al., 2013). However, the molecular information of the components of SOM fractions are still lacking in most studies (Kim et al., 2003). In the past decade, electrospray ionization (ESI) coupled with Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) has become a useful method because it offers unparalleled mass resolving power (>1 M) and mass measurement accuracy (<1 ppm). The organic compounds can be assigned to thousands of molecular formulas of known natural organic matter (Tfaily et al., 2015). In this way, it is possible to identify the chemical compositions of extremely complicated samples (Choi et al., 2017; Guigue et al., 2016; Wu et al., 2018). FT-ICR-MS has to date been proven feasible in distinguishing organic compounds in both terrestrial and aquatic environments, such as paddy soil and sediments as well as groundwater (Ajaero et al., 2017; Herzsprung et al., 2016; Li et al., 2018; Tfaily et al., 2015; Zhang et al., 2016).

71 The process of SOM decomposition should share some relationship to the
72 microbial community that catalyzes its formation, e.g. the microbial taxa that respond
73 differently to the different incorporated organic materials (Zheng et al., 2018).
74 Generally, easily degradable C compounds have a higher proportion of common
75 microbial taxa responsible for its mineralization (Yan et al., 2018). In this regard, we
76 hypothesized that different agronomic strategies, such as corn stover retention and
77 cover crop, because of their very different C constituents at the time of C return to soil,
78 would result in differences in soil bacterial community structure as well as in the
79 enrichment of chemical classes of extractable SOM. To test this, we sampled a field
80 experiment with treatments of corn stover retention, rye cover crop, both, and neither at
81 the W.K. Kellogg Biological Station (Michigan, USA). The objectives were to unravel:
82 1) the extent to which the corn stover and cover crop influence SOM and the identities
83 of its extractable components; 2) the responsiveness of bacterial and fungal
84 communities to the four treatments and 3) whether there are SOM compounds
85 indicative of different agronomic practices. This knowledge will improve our
86 understanding of the factors regulating the soil microbial community in a biofuel or
87 similarly managed agricultural ecosystem.

88 **2. Material & Methods**

89 *2.1. Field sites and sample overview*

90 The experiment site is located at the Great Lakes Bioenergy Research Center
91 (GLBRC) Biofuels Cropping System Experiment (BCSE) at Kellogg Biological

92 Station in southwest Michigan, USA. The BCSE experimental design utilizes a
93 randomized complete block design with 5 replicate blocks (30 × 40 m). The site had the
94 same or very similar cropping history prior to establishing the current plot design in
95 2008. The soil is predominantly a Kalamazoo loam (Fine-Loamy, Mixed, Semiactive,
96 Mesic Type Hapludalf), with 47-56% sand (Austin et al., 2017). There are a total of ten
97 biofuel cropping systems as described previously (Zhang et al., 2017) (referred to
98 https://lter.kbs.msu.edu/maps/images/20170316-glbrc-kbs-bcse-map.pdf), of which
99 two corn systems were selected for current study: continuous corn (*Zea mays*) (G1) and
100 continuous corn + rye cover crop (*Secale cereal)* (G2). For each replicate in G1 system,
101 the corn stover in main plot was removed after harvest (control), and in the subplot the
102 corn stover was left in the field (denoted as CS). For each replicate in the rye cover crop
103 G2 system, the corn stover was removed in the main plot (denoted as CC), and all of the
104 stover was left in the subplot (denoted as CSCC). The cover crop was harvested just
105 prior to corn planting in the spring and its aboveground biomass removed as part of the
106 treatment's biofuel yield. The subplots were established for the 2012 season so had
107 experienced 5 years treatment different from the main plots which had experienced 9
108 years of their treatments.

109 Soil samples were taken February 20, 2017, during a winter soil thaw. Soil
110 temperature was below 4^{o}C limiting current microbial growth. Five randomly
111 distributed cores (2-cm diameter) of 10 cm depth were composited to form a replicate
112 in each plot/subplots, and there were five replicates collected for each treatment. Soil

113 samples were then put into a container of dry-ice immediately and transported to the
114 laboratory within 3 h after collection. In the laboratory, samples were divided into two
115 portions: one portion was air-dried for determination of total SOM content. The other
116 was frozen at -80^{o}C for later SOM chemical composition characterization,
117 phospholipid fatty acid (PLFA) determination, and DNA extraction.
118 On November 22, 2017, a month after the harvest/retention of corn stover and the
119 planting of rye, we collected a second set of soil samples from the same subplots/plots
120 following the identical procedure for a repeated determination of total SOM content.
121 This timing was chosen to represent the un-frozen stage of soil and to minimize the
122 influence of the fresh C input.
123 *2.2. Soil total organic matter*
124 The air-dried soil samples were sent to Michigan State University Soil and Plant
125 Nutrient Laboratory to determine the SOM content using the K_2CrO_4 external heating
126 method (http://extension.missouri.edu/explorepdf/specialb/sb1001.pdf).
127 *2.3. Solvent extraction for SOM*
128 A portion of the frozen soil samples were sent to Environmental Molecular
129 Sciences Laboratory (EMSL) at Pacific Northwest National Laboratory (Richland, WA,
130 USA) for molecular determination of SOM components.
131 Previously, different sequential extraction protocols have been compared to
132 reveal SOM fractions by Tfaily et al (2017). Afterwards, an optimized sequential
133 protocol, i.e. water, methanol (MeOH), and chloroform ($CHCl_3$), is adopted by Graham

et al. (2017) for the river sediments. In this study, the identical sequential extraction protocol was employed for agricultural soil samples. According to Tfaily et al (2015; 2017), each solvent is selective toward specific types of compounds. Water is the natural in situ extractant with a selection bias for carbohydrates with high O/C ratios, amino sugars, and other labile polar compounds. The water-extracted organic matter represents the most labile fraction of SOM and approximates the dissolved organic matter found in soil solution (Ohno et al., 2010). $CHCl_3$ is selective for nonpolar lipids associated with mineral interactions and cellular membranes (i.e., physically bound OC). MeOH has a polarity in between that of water and $CHCl_3$, and it thus extracts both water-soluble and bound-OC pools. The compositional overlap exists between water-soluble and MeOH extracted OC pools (Tfaily et al. 2015; Graham et al., 2017). The samples were prepared by adding 1 mL of solvent to 1 to 100 mg bulk soil and shaking in 2 mL capped glass vials for 2 h on an Eppendorf Thermomixer. Samples were removed from the shaker and left to stand before spinning down and pulling off the supernatant to stop the extraction. The soil residue was dried with nitrogen gas to remove any residual solvent, and then solvent MeOH and solvent $CHCl_3$ were added sequentially.

2.4. FT-ICR-MS Data Acquisition and Data Analysis

Molecular compositions of SOM in the soil extracts were determined by a 12 Tesla FT-ICR-MS (Bruker Daltonics Inc., Billerica, MA, USA), a DOE-BER national user facility located at EMSL.

155 The detailed process has been described by Tfaily, et al. (2015) and Graham et al.
156 (2017). Briefly, samples were introduced to the electrospray ionization source equipped
157 with a fused silica tube through a syringe pump with the optimal parameters established
158 in earlier dissolved organic matter (DOM) characterization experiments (Tfaily et al.,
159 2015). All sample peak lists for the entire dataset were aligned to each other prior to
160 formula assignment to facilitate consistent peak assignments and eliminate possible
161 mass shifts that would impact formula assignment. Putative chemical formulas were
162 assigned using the Compound Identification Algorithm (CIA) described by Kujawinski
163 et al. (2006). Chemical formulas were assigned based on the following criteria: S/N > 7,
164 and mass measurement error <1 ppm, taking into consideration the presence of C, H, O,
165 N, S and P and excluding other elements (Tfaily et al., 2017).
166 FT-ICR-MS m/z intensities were converted into presence/absence data prior to
167 analysis because differences in m/z intensity are influenced by ionization efficiency as
168 well as relative abundance (Graham et al. 2017). This approach avoids biases incurred
169 by different ionization efficiencies for different types of compounds and potential
170 interferences between compounds or from complexation with metals (Boye et al.,
171 2017). Then, from the formula assignment, the number of peaks of each class, i.e. the
172 relative abundance, was calculated and used for the downstream analysis. Note that the
173 relative abundance was used here as that is the convention used by chemists for this
174 type of data but in the biological sense it can be viewed as a richness, i.e., the number of
175 compounds.

To interpret the large data set, van Krevelen diagram is often plotted on the basis of the molar H/C ratios (y axis) and molar O/C ratios of the assigned compounds (Kim et al., 2003). It provide a means to compare the average properties of SOM and enable identification of the major biochemical classes. Compounds with similar structural characteristics fall within the same region within a van Krevelen plot (Zhang et al., 2016). For this study, the chemical compounds were grouped into the eight main families: lipids ($0 < O/C \leq 0.3$, $1.5 \leq H/C \leq 2.5$), proteins ($0.3 < O/C \leq 0.55$, $1.5 \leq H/C \leq 2.3$), amino sugars ($0.55 < O/C \leq 0.7$, $1.5 \leq H/C \leq 2.2$), carbohydrates ($0.7 < O/C \leq 1.5$, $1.5 \leq H/C \leq 2.5$), unsaturated hydrocarbons ($0 \leq O/C \leq 0.125$, $0.8 \leq H/C \leq 1.5$), lignin ($0.125 < O/C \leq 0.65$, $0.8 \leq H/C < 1.5$), tannins ($0.65 < O/C \leq 1.1$, $0.8 \leq H/C < 1.5$), and condensed hydrocarbons ($0 \leq O/C \leq 0.95$, $0.2 \leq H/C \leq 0.8$).

In this study, pairwise comparison was carried out between two contrasting treatments in order to show the effect on SOM compositions of corn stover (i.e. control vs CS, and CC vs CSCC) and cover crop (control vs CC, and CS vs CSCC). For each paired group, one treatment was referred to the "counterpart" of the other. We defined the "unique" SOM compounds based on the following criterion: the chemical formulas that appeared in all the five replicates of a treatment but none of the other. Then, the "unique" SOM compounds were visualized on van Krevelen diagrams. In this way, we could highlight the types of compounds that enriched or disappeared between the paired treatments.

196 Aromaticity index (AI) is an indication of the refractory behavior of molecules
197 against biodegradation (Guigue et al., 2016). It considers the possibility that
198 heteroatoms (in particular O) can form double bonds not contributing to the aromaticity
199 (Koch and Dittmar, 2006). Less aromatic compounds reflect potentially higher
200 biodegradability of SOM (Choi et al., 2017). In this study, AI was employed to interpret
201 the presence of aromatic structures in a molecule after pooling all the chemical
202 formulas of SOM from three solvents together. We calculated the AI from the number
203 of atoms according to Eqn. (1):

204 $$AI = (1+C-O-S-0.5H)/(C-O-S-N-P) \quad (1)$$

205 Where C, O, S, H, N, and P represented the number of C, O, S, H, N, P atoms of
206 each compound, respectively (Koch and Dittmar, 2006). The compounds with values of
207 AI>0.5 are expected to be aromatic species (Choi et al., 2017).

208 *2.5. Phospholipid fatty acid (PLFA) analysis*

209 A portion of freeze-dried soil samples were analyzed for PLFAs by Microbial ID,
210 Inc.; (MIDI, Newark, DE, USA). Methods for extraction were adapted from company's
211 standard procedure.

212 Individual fatty acid methyl esters (FAMEs) were identified and quantified using
213 the MIDI Sherlock Microbial Identification System (MIDI, Newark, Delaware, USA).
214 The result for each individual fatty acid was expressed as a percentage of the total
215 amount of fatty acids (mol%) found in a given sample. The combined masses of
216 FAMEs reported as typical of fungi (18:2ω6c), general bacteria (15:0; i15:0; a15:0;

217 i16:0; 16:0ω9; i17:0; a17:0; cy17:0; 18:1ω7; cy19:0), Gram-negative bacteria (14:0;
218 16:1ω6c;17:1ω8c; 18:1ω7c; cy17:0; cy19:0; 20:1ω9c; 21:1ω8c; 21:1ω3c; 22:1ω3c),
219 Gram-positive bacteria (15:1ω9c; 15:1ω6c; 15:1ω9c; i15:0; a15:0; 16:0; 17:1ω9c;
220 a17:0; 18:0; 17:1ω9c), Actinobacteria (10Me16:0; 17:1ω7c; 10Me17:0; 18:1ω7c;
221 10Me18:0), arbuscular mycorrhyzal fungi (AMF; 16:1ω5c) were used as signatures for
222 these microbial groups. Fungal and bacterial markers were used to calculate fungal to
223 bacterial biomass ratio (F:B).

224 *2.6. DNA extraction, PCR amplification, and sequencing*

225 Soil microbial DNA was extracted from the frozen soil samples using PowerMax
226 Soil DNA Isolation Kit (MO BIO, CA, USA) following the manufacturer's protocol
227 and quantified using the Qubit dsDNA HS Assay Kit (Life Technologies, OR, USA)
228 with a Qubit fluorometer (Invitrogen, OR, USA). The extracted DNA was used for
229 quantitative analysis and for 16S rRNA gene amplicon sequencing.

230 *2.7. 16S rRNA gene amplicon sequencing and data analysis*

231 The V4 region of the 16S rRNA genes was sequenced with a one-step PCR library
232 preparation strategy. Briefly, the PCR was carried out with the target-only primer pair
233 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R
234 (5'-GGACTACHVGGGTWTCTAAT-3') (Caporaso et al., 2011). In the second-round
235 PCR, phasing primers with Illumina functionalities, spacers, as well as barcodes on the
236 reverse primers were introduced. Sample libraries were generated from purified PCR
237 products and pooled for sequencing as reported previously (Zhang et al., 2017).

238 To control variation resulting from an unequal number of sequences across
239 samples, sequence resampling was performed for each sample after OTU generation at
240 a rarefication sequence level based on the sample with the fewest number of sequences.
241 Sequences from each sample were randomly drawn from the original pool until the
242 rarefication sequence level is achieved. Once a sequence is drawn, it is excluded from
243 further rounds of selection to prevent repetition.
244 After sequencing was completed, 16S rRNA gene data were processed using the
245 Quantitative Insights Into Microbial Ecology (QIIME) pipeline using default
246 parameters unless otherwise noted (Caporaso et al., 2010). Reads below a quality score
247 of 25 and 200 bp in length were trimmed, and then assigned to soil samples based on
248 unique 5-bp barcodes. Operational Taxonomic Units (OTUs) were selected using the
249 UPARSE pipeline with a sequence similarity cut-off of 97% (USEARCH software V8)
250 (Edgar, 2017); chimeric sequences were also removed. Taxonomy was assigned to
251 OTUs against a subset of the Silva 119 database
252 (http://www.arb-silva.de/download/archive/qiime/) using PyNAST. The 16S rRNA
253 gene sequencing data have been deposited at DNA Data Bank of Japan (DDBJ) under
254 accession number DRA010263.
255 *2.8. Statistical analysis*
256 One-way analysis of variance (ANOVA) followed by Tukey's HSD test was used
257 to determine the effects of corn stover and cover crop on SOM content, 16S rRNA gene
258 copy number, PLFAs, and the relative abundances of bacterial community

compositions among different treatments. Significant differences were accepted at $P < 0.05$.

For the changes in soil bacterial community and SOM structure, the Bray-Curtis distances were computed based on the OTU tables and the total amount of SOM biochemical classes, and were then visualized with non-metric multidimensional scaling (NMDS) plots using the "metaMDS" function (vegan package) in R (Version 3.6.0). Permutational multivariate analysis of variance (PERMANOVA) was conducted to detect pairwise differences in group distances by using "adonis" function (vegan package, 999 permutations). The relative importance of the SOM compounds dissimilarities contributing to the variation in the soil bacterial taxonomic compositions (OTU level) was further identified by redundancy analysis (RDA) and a subsequent Monte Carlo test (999 permutations), which was performed using "rda" and "permutest" function of vegan package (Bray-Curtis distances). The goodness-of-fit (R^2) and associated statistical significance (P-value) of each SOM compound were verified using "envfit" function in vegan.

3. Results

3.1. SOM content

The total SOM content varied from 1.6% to 3.4%, with the average values listed in Table 1. The highest value was found in CSCC and the lowest in the control, although the P-value for comparisons among treatments just exceeded usual statistical thresholds

279 for significance (ANOVA, P=0.051). Similar trends were observed for the November
280 samples in the same year of 2017.

281 *3.2. Differences in number of components extracted by the three solvents*

282 The richness of compounds (peak number) varied with the extracting solvents
283 and organic amendments as well. Generally, $CHCl_3$ extracted more SOM compounds
284 than MeOH and water (Fig 1). In the water extracts, the control had the most peaks
285 (1,110), followed by CS (915) and CC (897). The lowest number of peaks was observed
286 in CSCC (637), significantly different from the control as well as CS and CC (P=0.001).
287 It was further found that the relative abundances of chemical classes decreased in the
288 order: condensed hydrocarbons>lignin>lipids>proteins (Fig S1).In the MeOH extracts,
289 no significant differences were observed among treatments (P=0.215, Fig 1), and the
290 most abundant class was lipid, followed by protein and lignin (Fig S1). For the $CHCl_3$
291 extracts, only CSCC (2,250) had significantly fewer peaks in comparison with the
292 control (2,920, P=0.01). Similar to the distribution in the MeOH extracts, lipid was the
293 most abundant (Fig S1).

294 *3.3. Aromaticity compounds*

295 The percentages of aromatic compounds (AI>0.5) varied substantially in different
296 extracts, with the average highest in water, followed by MeOH and $CHCl_3$ (Fig 2), in
297 line with each solvent's extractive traits. Different agronomic practices produced
298 differences in the proportions of AI>0.5 compounds. CS significantly decreased the
299 proportion of AI>0.5 compounds compared to the control (P<0.05) in water and MeOH.

300 When cover crop present, there was no significant difference due to stover retention,
301 CC vs CSCC. By contrast, the cover crop's presence with water extracts showed an
302 increasing proportion of AI>0.5 when compare to the control, as well as with stover,
303 CSCC vs CS ($P<0.05$).

304 *3.4. Unique SOM compounds*

305 Van Krevelen plots, together with Venn diagrams, were employed to show the
306 SOM compound characteristics (Fig 3 and Fig S3). Among all treatments, the
307 common compounds only accounted for 19.8%, 18.4%, and 0.1% in the water, MeOH,
308 and $CHCl_3$ extracts, respectively (Fig S2). The unique compounds varied with the
309 solvents and the organic amendments. Specifically, in the water extracts, the unique
310 compounds in the control accounted for 17.4% of total compounds, larger than other
311 treatments, and those in CSCC accounted for the least (0.4%). In the MeOH extracts,
312 large proportions of unique compounds were observed in CS and CSCC (28.7% and
313 29.4%, respectively), while those in the control and CC accounted for only 3.8% and
314 0.6%, respectively. In the $CHCl_3$ extracts, the proportions of unique compounds
315 ranged from 3.9% (control) to 25.6% (CS).

316 Pairwise comparison between the contrasting treatments showed that the unique
317 SOM compounds varied depending on treatments and extracting solvents, as illustrated
318 by van Krevelen diagrams and the derived stacked plots (Fig 3 and Fig S4). Specifically,
319 in the water extracts (Fig 3-a), the augmented treatment (CS or CC) generated fewer
320 compounds in comparison with the control, which contained a broad range of

321 compounds, including protein-, lignin-, and carbohydrates-like compounds. CS
322 appeared to have some unique lignin-like compounds, while CC led to the enrichment
323 of condensed hydrocarbons.

324 In the MeOH extracts, it was notable that no unique compound appeared in CS or
325 CC as compared to control that harbored various unique ones (Fig 3-b1 and 3-b3). By
326 contrast, CSCC produced more kinds of unique compounds, including lipids, proteins,
327 and carbohydrates, in comparison with either CS or CC (Fig 3-b2 and 3-b4), suggesting
328 that CSCC had an enhancing effect.

329 In the $CHCl_3$ extracts, the most abundant unique compounds were lipid- and
330 protein-like, in agreement with the solvent trait (Fig 3-c). However, the pattern of
331 unique compounds produced by corn stover diverged from that by cover crop. For
332 example, CS harbored more unique compounds than the control (Fig 3-c1), a similar
333 trend observed between CSCC and CC (Fig 3-c2). By contrast, CC possessed less
334 unique compounds than the control (Fig 3-c3), as well as CSCC compared to CS (Fig
335 3-c4).

336 NMDS analysis based on the chemical classes of SOM from three solvents
337 showed that only CSCC separated from other treatments (Fig 4, PERMANOVA,
338 P=0.045), reflecting the strongest effect of coupled application of stover retention and
339 cover crop.

340 *3.5. Microbial and functional group lipid biomass*

PLFA analysis results showed that most of the microbial and functional group lipid biomass were generally similar among treatments, including total biomass, eukaryote, fungi, and bacteria (Table 2). Lipid marker for arbuscular mycorrizhal fungi (AMF) was significantly higher in CSCC (4.53%) and CC (4.57%) than in control (4.12%). Considering that the soil was dominated by bacteria rather than fungi (F/B ratio was 0.065-0.072), we focused on the bacterial community in the follow-up analysis.

3.6. Bacterial population in soil

Real-time qPCR results showed that the bacterial population in soil ranged from 2.1×10^{9} to 8.95×10^{9} gene copy number per gram (Table 2), although no significant differences were found among all treatments according to the one-way ANOVA (Tukey's test, P=0.526).

3.7. Soil bacterial community composition

Taxonomic analysis of 16S rRNA gene amplicons showed that Proteobacteria was the most abundant phylum (23.2-34.1%), followed by Acidobacteria (10.56-25.8%), Actinobacteria (3.55-11.3%), Verrucomicrobia (4.97-12.39%), Bacteroidetes (3.67-13.29%), Planctomycetes (2.15-7.09%), Gemmatimonadetes (1.15-2.88%), and Firmicutes (0.43-3.26%). Several phyla were shifted by the amendments in term of their relative abundances (Fig 5). For example, a significantly lower proportions of Acidobacteria (P=0.001), Nitrospirae (P=0.014), and Armatimonadetes (P=0.008)

361 were found in CSCC, compared to the control. Further, CS had a significantly lower
362 abundance of Actinobacteria than the control (P=0.045).
363 At the finer levels, some lineages had distinct responses to different field
364 management (Table 3). In particular, CSCC decreased significantly the occurrences of
365 Acidobacteria GP6 (P=0.041), Clostridia (P=0.001), and Nitrospira (P=0.009), but
366 significantly increased those of Sphingobacteria (P=0.043) and Thermomicrobia
367 (P=0.009) in comparison with the control. By contrast, CS increased significantly the
368 proportion of Myxococcales (P=0.021), but significantly decreased that of Clostridia
369 (P=0.041) as compared to the control.
370 Non-metric multidimensional scaling (NMDS) ordination at the OTU level
371 (Bray-Curtis dissimilarity matrices) demonstrated the discrepancy of the bacterial
372 communities among treatments (Fig S4). Especially, distinct separations were found
373 between control vs CS (R^2=0.22, P=0.03), control vs CSCC (R^2=0.32, P=0.03), and CS
374 vs CSCC (R^2=0.32, P=0.042).
375 *3.8. Relationship between soil bacterial community and SOM compounds*
376 The RDA biplot (Fig 6) showed that the SOM compounds in all the extracts
377 explained 38.5% of variability in the bacterial community composition. The canonical
378 coefficients, the goodness-of-fit (r^2) and associated statistical significance (P-value) of
379 each SOM compound is in Table S1. Along the axis 1, some $CHCl_3$-extracted
380 unsaturated hydrocarbons (r^2=0.63, P=0.001) and lipids (r^2=0.37, P=0.020), were
381 significantly correlated with CSCC, while some $CHCl_3$-extracted lignin (r^2=0.41,

382 P=0.014) and proteins (r^2=0.38, P=0.019) had significantly positive relationship with
383 CS.

384 **4. Discussion**

385 In this study, the amendments of stover retention and cover crop, singly and in
386 combination, did not lead to significant changes in total SOM content after 5-9 years
387 but positive trends were apparent, especially versus the control. This sluggish
388 response is in accordance with some previous studies. Examples include a 13-year
389 addition of a winter cereal rye cover crop at three locations in Maryland, USA (Steele et
390 al., 2012), and the study of retaining residue in the short term (<10 years)
391 (Blanco-Canqui and Lal, 2009). However, there are also some findings showing gains
392 in SOM contents after the organic amendments (Urra et al., 2018; Warren Raffa et al.,
393 2015; Wegner et al., 2018). We assumed such divergences might derive from the
394 edaphic properties, climates, amending strategy, and management history.

395 *4.1. Organic amendments decreased the richness of SOM compounds and changed the*
396 *occurrence of unique compounds*

397 Increasing organic matter inputs generally increases soil organic matter content
398 (West and Six 2007). If organic matter inputs increase microbial activity, we expect
399 that the increased microbial biomass and necromass will contribute to soil organic
400 matter formation (Six et al. 2006), with some of it contributing to the stable mineral
401 fraction (Cotrufo et al. 2015). In this sense, we hypothesized that an increase in SOM
402 would be accompanied by more unique SOM compounds. Surprisingly, contrary

403 results were found especially in water and MeOH extracts (Fig 2 and Fig 3). This
404 outcome is presumably the result of microbial activity and community selection over
405 the decade of treatments. We offer two explanations, perhaps related. First, the fresh
406 organic treatments would provide a priming effect, thereby stimulating the degradation
407 of current and some of the old C compounds leaving less to be extracted
408 (Blagodatskaya and Kuzyakov, 2008; Pegoraro et al., 2019). Second, the 5-9-year
409 selection of copiotrophs on the new C produced metabolites common in soil (i.e. less
410 unique SOM compounds). By contrast, the old, recalcitrant organic compounds in
411 non-amended soil served as the main C and energy sources for microorganisms
412 (especially oligotrophs), resulting in diverse metabolites or more persistent residues (i.e.
413 more unique SOM compounds). This is supported by a previous study that confirms the
414 depletion of recalcitrant SOM fractions in stover-removed soil based on quantitative
415 ^{13}C NMR (Stetson et al., 2012), a sign of the role of recalcitrant SOM compounds for
416 microorganisms. Also, the increased proportion of AI>0.5 compounds in the control of
417 this study (Fig 2) is consistent with such a process, because the degradation of aromatic
418 ring cleavage would lead to an increase of aromatic C and/or condensed aromatic
419 structures (Derrien et al., 2017).

420 *4.2. Stover retention and cover crop influenced SOM compounds differently*

421 For a given site with similar soil type, the initial quality of organic materials is
422 intimately related to their decomposition process. Generally, corn stover has a higher
423 C/N ratio (e.g. 79.5 reported by Yang et al. (2017)) than rye cover crop (e.g. 22.2 by

424 Barel et al. (2018)). Such differences will lead to the variance in aromaticity and the
425 unique SOM compounds, as revealed by pairwise comparison in this study (Fig 2).
426 Stover, representing the dead plant residue, led to fewer aromatic compounds (Fig
427 2). This phenomenon implied that the less humified pools of soil C are not being
428 replenished in the stover retention treatment (Stetson et al., 2012). Similar trends are
429 observed in other studies (Chen et al., 2017; Song et al., 2017). In addition, according to
430 the van Krevelen plots, the unique compounds generated by stover retention were
431 primarily lignin-like (in water and $CHCl_3$ extracts) and lipid-like (in $CHCl_3$ extracts)
432 (Fig 3). Lignin-like compounds are likely derived from allochthonous terrestrial plant
433 sources, and represent the bulk of the semi-labile and refractory SOM pool (Lusk et
434 al., 2016). By contrast, lipid-like compounds are aliphatic and microbially derived,
435 and typically represent the bulk of the labile pool (Hendrickson et al., 2007). Two
436 different pathways/stages of SOM formation are proposed by Cotrufo et al. (2015)
437 after litter incorporation. At the early stage, the non-structural compounds are lost from
438 the litter, and the acid un-hydrolysable fraction (generally defined as 'lignin') increases
439 in absolute amounts (though different from the types of SOM in this study), owing to
440 the incorporation of microbial residues in this fraction. At the late stage, physical
441 transfer of the residue to the mineral soil takes effect, resulting in no preferential loss
442 of any chemical compounds and no incorporation of microbially produced residue C.
443 In this respect, we suggest that early stage SOM formation is occurring in this study

444 ~3 months after stover retention. At this stage, microbial decomposition produces
445 necromass which includes refractory structures like lipids and mineral bound lipids.
446 On the contrary, cover crop tended to influence SOM compounds in a different
447 way, i.e., the increased proportion of AI>0.5 compounds and the emergence of unique
448 condensed hydrocarbons (Fig 2 and Fig 3). We presume that this is owing to the
449 involvement of plant activity, i.e. the rhizosphere effect. In rhizosphere samples,
450 compounds with aromatic structures and less aliphatic are more abundant than in
451 non-rhizosphere samples, resulting in a more recalcitrant C pool (Wen et al., 2018). A
452 previous study also shows the increased proportion of AI>0.5 compounds by rye cover
453 crop based on diffuse reflectance Fourier transform infrared (DRIFT) spectra (Ding et
454 al. 2006), which was in good agreement with current study, particularly in the water
455 extracts (Fig 2). Further illustration of unique SOM compounds (Fig 3) unraveled that
456 cover crop garnered more condensed hydrocarbons (water extracts) and lipids ($CHCl_3$
457 extracts), which, we assume, were also intimately associated with the plant, especially
458 root exudates.
459 *4.3. Microorganisms' response to stover retention and cover crop*
460 Neither stover retention nor cover crops changed the total microbial biomass,
461 bacterial biomass or 16S rRNA gene copy number as revealed by PLFA and qPCR.
462 However, some functional microbial groups were altered. For example, the lipid
463 biomarker of AMF had significantly higher biomass in the cover crop treatments (CC
464 and CSCC), rather than CS, compared with that in the control (Table 2). This was

consistent with the increasing trend of SOM content above (Table 1). Presumably, such differences resulted from the traits of organic materials. Rye cover crop favors the proliferation of AMF (García-González et al., 2018), which subsequently contributed to the increase in soil C storage due to their extensive mycelium and necromass in the presence of rye roots (Zhu and Michael Miller, 2003). Besides, the yearly input of root biomass from rye cover crop is nearly 44.8 g C m^{-2} (Austin et al. 2017), which should be sufficient to sustain a large AMF community.

Further exploration on bacterial community showed the greatest impact of CSCC on the bacterial compositions, compared to either CS or CC individually (Fig 5 and Table 3). We ascribe this enhanced effect of CSCC to the fact that CSCC harbored both living and dead organic materials. In CSCC, some lineages that might prefer degrading those materials were enriched, such as Burkholderiales (Betaproteobacteria) and Sphingobactertia (Bacteroidetes), while some K-strategists (oligotrophs) were constrained, such as Acidobacteria Gp6. Some Burkholderiales have the ability to decompose high molecular weight organic compounds (Gu et al., 2017), and Sphingobacteriia can utilize lignocellulosic material for growth (Yan et al., 2012), while Acidobacteria, in all likelihood, degrade ancient or recalcitrant SOM in nutrient-poor conditions (Wang et al., 2018). Since the bacterial structure of CSCC in this study separated from other treatments by $CHCl_3$ extracted lipids as revealed by RDA biplot (Fig 6), and lipids could come from microbes and not just plants, it might

485 be inferred that combining both treatments increases lipid abundance due to both plant
486 and microbe inputs.

487 Despite the negligible shifts in the whole bacteria community (Fig S4), CS
488 increased the relative abundances of some lineages, such as Myxococcales
489 (Deltaproteobacteria), but decreased that of Actinobacteria. Myxobacteria, ubiquitous
490 in soil environments, are predators and can access nutrients from a broad spectrum of
491 microorganisms (Thiery and Kaimer, 2020). Actinobacteria are a key bacterial group in
492 the utilization of readily available C. The opposite trends of those groups might imply
493 an inverse relationship between them. It is noteworthy that the bacterial communities in
494 stover-contained treatments (CS and CSCC) were significantly correlated to
495 $CHCl_3$-extracted components, i.e. proteins and lignin in the $CHCl_3$ extracts with CS,
496 and lipids and unsaturated hydrocarbon in the $CHCl_3$ extracts with CSCC (Fig 6). This
497 feature suggested that in stover-contained treatments the bacteria preferred the
498 mineral-associated SOM fractions, and further supported the necessity of using
499 sequential extraction protocols, rather than water solvent only, to exhaustively depict
500 the non-polymer SOM compounds. By contrast, no significant change in the bacterial
501 community was observed for the single cover crop treatment, suggesting a different
502 microbial selection in the corn stover soil.

503 Soil C stocks change slowly, usually on decadal time scales (Deng et al. 2016),
504 but soil C characteristics can change more rapidly. For example, the proportion of C
505 in the active versus passive pools can change after just a few years of altered

506 management (Sprunger and Robertson 2018). Here, we show that soil organic C
507 composition and bacterial communities are also altered by changing inputs. Will this
508 lead to increased total soil organic C? Perhaps. At our field site, the organic
509 amendments lead to higher soil organic C on average (although not statistically
510 significant), suggesting a trend toward higher C accumulation with cover crops and
511 stover retention. In addition, the cover crop treatment increased the presence of
512 aromatic compounds, and the stover increased the presence of lignin, which are both
513 recalcitrant compounds. This trend may not be universal, however. The coarse texture
514 and medium C concentration at our site are more likely to accumulate C than
515 fine-textured soils or those with high clay and soil organic matter content (West and
516 Six 2007; Johnston et al. 2009; Sprunger and Robertson 2018; Szymanski et al. 2019).
517 Similarly, it is not yet clear how shifts in microbial community composition will
518 influence soil C accumulation. We observed the microbial community shift in parallel
519 with soil C chemistry, possibly in a way that optimizes C consumption (see previous
520 paragraphs in this section). As a result, these microbial community changes could lead
521 to 1) increases in C mineralization that offset the additional C inputs, or 2) increased
522 microbial biomass and necromass, which increases total soil organic C (Six et al.
523 2006; Cotrufo et al. 2015).
524 In this study we demonstrate the changes in SOM biochemical and microbial
525 structure that occur from different types of SOM amendments. Although our results
526 hint at mechanisms, we cannot yet link specific SOM structures to the soil bacterial

community due to technical limitations. Nonetheless, this paper is an important first step in understanding mechanisms of SOM formation. Further research could examine the long-term fate of specific C compounds, perhaps via isotopically-labeled additions of these compounds, coupled with microbial community assessments. FT-ICR-MS is a useful tool for examining the degradation of organic C and for understanding the underlying mechanisms of soil C formation and retention.

5. Conclusions

This study provides insights regarding the effects of corn stover retention and cover crop on SOM compounds and the soil microbial community. Although there were negligible variations in total SOM content, total microbial biomass, and bacterial population due to these C amendment practices, significant changes were found for some SOM chemical compounds, AMF biomass and some bacterial linages, with corn stover and cover crop showing different impacts on those parameters. Soil biosystems respond slowly to C management practices, with resultant soil changes occurring as a function of type of organic C addition, the microbiome, and the organic compounds added and their residual signatures.

Acknowledgements

Support for this research was provided by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research (Awards DE-SC0018409 and DE-FC02-07ER64494), by the National Science Foundation Long-term

Ecological Research Program (DEB 1637653) at the Kellogg Biological Station, by Michigan State University AgBioResearch, and by China Scholarship Council. A portion of the research was performed at the EMSL User Facility. We thanked all people who provide help but were not listed as authors at EMSL.

554 **References**

555 Ajaero, C., McMartin, D.W., Peru, K.M., Bailey, J., Haakensen, M., Friesen, V., Martz, R., Hughes,
556 S.A., Brown, C., Chen, H., McKenna, A.M., Corilo, Y.E., Headley, J.V., 2017. Fourier transform
557 ion cyclotron resonance mass spectrometry characterization of athabasca oil sand
558 process-affected waters incubated in the presence of wetland plants. Energy & Fuels 31,
559 1731-1740.

560 Austin, E.E., Wickings, K., McDaniel, M.D., Robertson, G.P., Grandy, A.S., 2017. Cover crop root
561 contributions to soil carbon in a no-till corn bioenergy cropping system. GCB Bioenergy 9,
562 1252-1263.

563 Barel, J.M., Kuyper, T.W., Paul, J., Boer, W., Cornelissen, J.H.C., De Deyn, G.B., Cheng, L., 2018.
564 Winter cover crop legacy effects on litter decomposition act through litter quality and microbial
565 community changes. Journal of Applied Ecology 56, 132-143.

566 Blagodatskaya, E., Kuzyakov, Y., 2008. Mechanisms of real and apparent priming effects and their
567 dependence on soil microbial biomass and community structure: critical review. Biology and
568 Fertility of Soils 45, 115-131.

569 Blanco-Canqui, H., Lal, R., 2009. Crop residue removal impacts on soil productivity and
570 environmental quality. Critical Reviews in Plant Sciences 28, 139-163.

571 Boye, K., Noël, V., Tfaily, M.M., Bone, S.E., Williams, K.H., Bargar, John R., Fendorf, S., 2017.
572 Thermodynamically controlled preservation of organic carbon in floodplains. Nature
573 Geoscience 10, 415-419.

574 Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N.,

Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7, 335-336.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N., Knight, R., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Sciences 108, 4516.

Cates, A.M., Jackson, R.D., 2019. Cover Crop Effects on Net Ecosystem Carbon Balance in Grain and Silage Maize. Agronomy Journal 111, 30-38.

Chen, X., Mao, A., Zhang, Y., Zhang, L., Chang, J., Gao, H., Thompson, M.L., 2017. Carbon and nitrogen forms in soil organic matter influenced by incorporated wheat and corn residues. Soil Science and Plant Nutrition 63, 377-387.

Choi, J.H., Kim, Y.-G., Lee, Y.K., Pack, S.P., Jung, J.Y., Jang, K.-S., 2017. Chemical characterization of dissolved organic matter in moist acidic tussock tundra soil using ultra-high resolution 15T FT-ICR mass spectrometry. Biotechnology and Bioprocess Engineering 22, 637-646.

Cotrufo, M.F., Soong, J.L., Horton, A.J., Campbell, E.E., Haddix, Michelle L., Wall, D.H., Parton, W.J., 2015. Formation of soil organic matter via biochemical and physical pathways of litter mass loss. Nature Geoscience 8, 776-779.

Deng, L., Zhu, G., Tang, Z., Shangguan, Z., 2016. Global patterns of the effects of land-use changes on soil carbon stocks. Global Ecology and Conservation 5, 127-138.

596 Derrien, M., Lee, Y.K., Park, J.E., Li, P., Chen, M., Lee, S.H., Lee, S.H., Lee, J.B., Hur, J., 2017.
597 Spectroscopic and molecular characterization of humic substances (HS) from soils and
598 sediments in a watershed: comparative study of HS chemical fractions and the origins.
599 Environmental Science and Pollution Research 24, 16933-16945.
600 Ding, G.W., Liu, X.B., Herbert, S., Novak, J., Amarasiriwardena, D., Xing, B.S., 2006. Effect of cover
601 crop management on soil organic matter. Geoderma 130, 229-239.
602 Edgar, R.C., 2017. SEARCH_16S: A new algorithm for identifying 16S ribosomal RNA genes in
603 contigs and chromosomes. bioRxiv.
604 García-González, I., Hontoria, C., Gabriel, J.L., Alonso-Ayuso, M., Quemada, M., 2018. Cover crops to
605 mitigate soil degradation and enhance soil functionality in irrigated land. Geoderma 322, 81-88.
606 Graham, E.B., Tfaily, M.M., Crump, A.R., Goldman, A.E., Bramer, L.M., Arntzen, E., Romero, E.,
607 Resch, C.T., Kennedy, D.W., Stegen, J.C., 2017. Carbon Inputs From Riparian Vegetation Limit
608 Oxidation of Physically Bound Organic Carbon Via Biochemical and Thermodynamic Processes.
609 Journal of Geophysical Research: Biogeosciences 122, 3188-3205.
610 Gu, Y.F., Wang, Y.Y., Lu, S.E., Xiang, Q.J., Yu, X.M., Zhao, K., Zou, L.K., Chen, Q., Tu, S.H., Zhang,
611 X.P., 2017. Long-term fertilization structures bacterial and archaeal communities along soil
612 depth gradient in a paddy soil. Frontiers in Microbiology 8.
613 Guigue, J., Harir, M., Mathieu, O., Lucio, M., Ranjard, L., Leveque, J., Schmitt-Kopplin, P., 2016.
614 Ultrahigh-resolution FT-ICR mass spectrometry for molecular characterisation of pressurised
615 hot water-extractable organic matter in soils. Biogeochemistry 128, 307-326.
616 Hendrickson, J., Trahan, N., Gordon, E., Ouyang, Y., 2007. Estimating relevance of organic carbon,

617 nitrogen, and phosphorus loads to a blackwater river estuary. Journal of the American Water
618 Resources Association 43, 264-279.
619 Herzsprung, P., Hertkorn, N., von Tuempling, W., Harir, M., Friese, K., Schmitt-Kopplin, P., 2016.
620 Molecular formula assignment for dissolved organic matter (DOM) using high-field
621 FT-ICR-MS: chemical perspective and validation of sulphur-rich organic components (CHOS)
622 in pit lake samples. Analytical and Bioanalytical Chemistry 408, 2461-2469.
623 Johnston, A.E., Poulton, P.R., Coleman, K., 2009. Chapter 1 Soil organic matter: its importance in
624 sustainable agriculture and carbon dioxide fluxes, In: Sparks, D.L. (Ed.), Advances in
625 Agronomy. Academic Press, pp. 1-57.
626 Kim, S., Kramer, R.W., Hatcher, P.G., 2003. Graphical method for analysis of ultrahigh-resolution
627 broadband mass spectra of natural organic matter, the van Krevelen diagram. Analytical
628 Chemistry 75, 5336-5344.
629 Koch, B.P., Dittmar, T., 2006. From mass to structure: an aromaticity index for high-resolution mass
630 data of natural organic matter. Rapid Communications in Mass Spectrometry 20, 926-932.
631 Kujawinski, E.B., Behn, M.D., 2006. Automated Analysis of Electrospray Ionization Fourier Transform
632 Ion Cyclotron Resonance Mass Spectra of Natural Organic Matter. Analytical Chemistry 78,
633 4363-4373.
634 Li, X., Sun, G., Chen, S., Fang, Z., Yuan, H., Shi, Q., Zhu, Y., 2018. Molecular chemodiversity of
635 dissolved organic matter in paddy soils. Environmental Science & Technology 52, 963-971.
636 Lusk, M.G., Toor, G.S., 2016. Dissolved organic nitrogen in urban streams: biodegradability and
637 molecular composition studies. Water Reserch 96, 225-235.

638 Martinez-Feria, R.A., Dietzel, R., Liebman, M., Helmers, M.J., Archontoulis, S.V., 2016. Rye cover
639 crop effects on maize: A system-level analysis. Field Crops Research 196, 145-159.
640 Ohno, T., He, Z., Sleighter, R.L., Honeycutt, C.W., Hatcher, P.G., 2010. Ultrahigh Resolution Mass
641 Spectrometry and Indicator Species Analysis to Identify Marker Components of Soil- and Plant
642 Biomass-Derived Organic Matter Fractions. Environmental Science & Technology 44,
643 8594-8600.
644 Pegoraro, E., Mauritz, M., Bracho, R., Ebert, C., Dijkstra, P., Hungate, B.A., Konstantinidis, K.T., Luo,
645 Y., Schädel, C., Tiedje, J.M., Zhou, J., Schuur, E.A.G., 2019. Glucose addition increases the
646 magnitude and decreases the age of soil respired carbon in a long-term permafrost incubation
647 study. Soil Biology and Biochemistry 129, 201-211.
648 Ruis, S.J., Blanco-Canqui, H., 2017. Cover Crops Could Offset Crop Residue Removal Effects on Soil
649 Carbon and Other Properties: A Review. Agronomy Journal 109, 1785-1805.
650 Sanchez, N.P., Skeriotis, A.T., Miller, C.M., 2013. Assessment of dissolved organic matter fluorescence
651 PARAFAC components before and after coagulation-filtration in a full scale water treatment
652 plant. Water Research 47, 1679-1690.
653 Six, J., Frey, S.D., Thiet, R.K., Batten, K.M., 2006. Bacterial and fungal contributions to carbon
654 sequestration in agroecosystems. Soil Science Society of America Journal 70, 555-569.
655 Song, G.X., Novotny, E.H., Mao, J.D., Hayes, M.H.B., 2017. Characterization of transformations of
656 maize residues into soil organic matter. Science of the Total Environment 579, 1843-1854.
657 Sprunger, C.D., Philip Robertson, G., 2018. Early accumulation of active fraction soil carbon in newly
658 established cellulosic biofuel systems. Geoderma 318, 42-51.

659 Steele, M.K., Coale, F.J., Hill, R.L., 2012. Winter annual cover crop impacts on no-till soil physical
660 properties and organic matter. Soil Science Society of America Journal 76, 2164-2173.
661 Stetson, S.J., Osborne, S.L., Schumacher, T.E., Eynard, A., Chilom, G., Rice, J., Nichols, K.A., Pikul,
662 J.L., Jr., 2012. Corn residue removal impact on topsoil organic carbon in a corn-soybean rotation.
663 Soil Science Society of America Journal 76, 1399-1406.
664 Stevenson, F.J., 1994. Humus Chemistry: Genesis, Composition, Reactions, 2nd ed. John Wiley &
665 Sons, Ltd, New York.
666 Stewart, C.E., Roosendaal, D.L., Manter, D.K., Delgado, J.A., Del Grosso, S., 2018. Interactions of
667 Stover and Nitrogen Management on Soil Microbial Community and Labile Carbon under
668 Irrigated No-Till Corn. Soil Science Society of America Journal 82, 323-331.
669 Szymanski, L.M., Sanford, G.R., Heckman, K.A., Jackson, R.D., Marín-Spiotta, E., 2019. Conversion
670 to bioenergy crops alters the amount and age of microbially-respired soil carbon. Soil Biology &
671 Biochemistry 128, 35-44.
672 Tfaily, M.M., Chu, R.K., Tolic, N., Roscioli, K.M., Anderton, C.R., Pasa-Tolic, L., Robinson, E.W.,
673 Hess, N.J., 2015. Advanced Solvent Based Methods for Molecular Characterization of Soil
674 Organic Matter by High-Resolution Mass Spectrometry. Analytical Chemistry 87, 5206-5215.
675 Tfaily, M.M., Chu, R.K., Toyoda, J., Tolić, N., Robinson, E.W., Paša-T, olić, L., Hess, N.J., 2017.
676 Sequential extraction protocol for organic matter from soils and sediments using high resolution
677 mass spectrometry. Analytica Chimica Acta 972, 54-61.
678 Thiery, S., Kaimer, C., 2020. The Predation Strategy of Myxococcus xanthus. Front Microbiol 11, 2.
679 Urra, J., Mijangos, I., Lanzén, A., Lloveras, J., Garbisu, C., 2018. Effects of corn stover management

on soil quality. European Journal of Soil Biology 88, 57-64.

Villamil, M.B., Bollero, G.A., Darmody, R.G., Simmons, F.W., Bullock, D.G., 2006. No-till corn/soybean systems including winter cover crops. Soil Science Society of America Journal 70, 1936-1944.

Wang, Q., Jiang, X., Guan, D., Wei, D., Zhao, B., Ma, M., Chen, S., Li, L., Cao, F., Li, J., 2018. Long-term fertilization changes bacterial diversity and bacterial communities in the maize rhizosphere of Chinese Mollisols. Applied Soil Ecology 125, 88-96.

Warren Raffa, D., Bogdanski, A., Tittonell, P., 2015. How does crop residue removal affect soil organic carbon and yield? A hierarchical analysis of management and environmental factors. Biomass and Bioenergy 81, 345-355.

Wegner, B.R., Osborne, S.L., Lehman, R.M., Kumar, S., 2018. Seven-Year Impact of Cover Crops on Soil Health When Corn Residue Is Removed. BioEnergy Research 11, 239-248.

Wen, J., Li, Z., Luo, N., Huang, M., Yang, R., Zeng, G., 2018. Investigating organic matter properties affecting the binding behavior of heavy metals in the rhizosphere of wetlands. Ecotoxicology and Environmental Safety 162, 184-191.

West, T.O., Six, J., 2007. Considering the influence of sequestration duration and carbon saturation on estimates of soil carbon capacity. Climatic Change 80, 25-41.

Wilke, B.J., Snapp, S.S., 2008. Winter cover crops for local ecosystems: linking plant traits and ecosystem function. Journal of the Science of Food and Agriculture 88, 551-557.

Wu, X., Wu, L., Liu, Y., Zhang, P., Li, Q., Zhou, J., Hess, N.J., Hazen, T.C., Yang, W., Chakraborty, R., 2018. Microbial interactions with dissolved organic matter drive carbon dynamics and

community succession. Frontiers in Microbiology 9.

Yan, J., Wang, L., Hu, Y., Tsang, Y.F., Zhang, Y., Wu, J., Fu, X., Sun, Y., 2018. Plant litter composition selects different soil microbial structures and in turn drives different litter decomposition pattern and soil carbon sequestration capability. Geoderma 319, 194-203.

Yan, L., Gao, Y., Wang, Y., Liu, Q., Sun, Z., Fu, B., Wen, X., Cui, Z., Wang, W., 2012. Diversity of a mesophilic lignocellulolytic microbial consortium which is useful for enhancement of biogas production. Bioresource Technology 111, 49-54.

Yang, X., Lan, Y., Meng, J., Chen, W., Huang, Y., Cheng, X., He, T., Cao, T., Liu, Z., Jiang, L., Gao, J., 2017. Effects of maize stover and its derived biochar on greenhouse gases emissions and C-budget of brown earth in Northeast China. Environmental Science and Pollution Research 24, 8200-8209.

Zhang, B., Penton, C.R., Xue, C., Quensen, J.F., Roley, S.S., Guo, J., Garoutte, A., Zheng, T., Tiedje, J.M., 2017. Soil depth and crop determinants of bacterial communities under ten biofuel cropping systems. Soil Biology & Biochemistry 112, 140-152.

Zhang, L., Wang, S., Xu, Y., Shi, Q., Zhao, H., Jiang, B., Yang, J., 2016. Molecular characterization of lake sediment WEON by Fourier transform ion cyclotron resonance mass spectrometry and its environmental implications. Water Research 106, 196-203.

Zheng, H., Chen, Y., Liu, Y., Zhang, J., Yang, W., Yang, L., Li, H., Wang, L., Wu, F., Guo, L., 2018. Litter quality drives the differentiation of microbial communities in the litter horizon across an alpine treeline ecotone in the eastern Tibetan Plateau. Scientific Reports 8, 10029.

Zhu, Y.G., Michael Miller, R., 2003. Carbon cycling by arbuscular mycorrhizal fungi in soil-plant

722 systems. Trends in Plant Science 8, 407-409.

Tables

Table 1 Total SOM contents in different amendments. Control, corn stover is removed after corn harvested. CS, corn stover retention. CC, both corn stover and cover crop are removed after corn harvested. CSCC, cover crop is removed but corn stover remains after corn harvested. Different letters within each column denote significant differences at $P<0.05$.

Treatment	SOM content (%)	
	February sample	November sample
control	2.12±0.33 a	1.84±0.29 a
CS	2.32±0.60 a	2.10±0.21 a
CC	2.48±0.37 a	2.52±0.13 a
CSCC	2.64±0.54 a	2.60±0.43 a

Table 2 The proportions of lipid groups in different treatments as determined by lipid analysis and 16S rRNA gene copy number determined by qPCR. Control, corn stover is removed after corn harvested. CS, corn stover retention. CC, both corn stover and cover crop are removed after corn harvested. CSCC, cover crop is removed but corn stover remains after corn harvested. Different letters within each column denote significant differences at $P<0.05$.

	TB (nmol/g)	AMF %	Eukaryote %	Fungi%	Actinobacteria %	F/B	Fungi	Bacteria	gene copies*10^9/g
control	69.57a	4.12b	1.32a	1.06a	18.08a	0.070a	6.51a	93.49a	5.92a
CS	64.60a	4.22ab	1.62a	0.73a	17.24a	0.065a	5.78a	93.98a	3.67a
CC	69.68a	4.57a	1.36a	0.92a	17.72a	0.072a	5.76a	92.89a	4.30a
CSCC	64.41a	4.53a	1.38a	0.87a	17.58a	0.064a	5.31a	93.87a	4.15a

TB, Total Biomass; AMF, Arbuscular Mycorrhiza Fungi; F/B, Fungi/Bacteria.

738 **Table 3** The relative abundances of the different lineages in soil bacterial community.

739 Control, corn stover is removed after corn harvested. CS, corn stover retention. CC,

740 both corn stover and cover crop are removed after corn harvested. CSCC, cover crop is

741 removed but corn stover remains after corn harvested. Different letters within each

742 column denote significant differences at $P<0.05$.

	Actinobacteria(%)	Acidobacteria Gp6(%)	Clostridia(%)	Nitrospirales(%)	Thermomicrobia(%)	Sphingobacteriia(%)	Burkholderiales(%)	Myxococcales(%)
control	13.15 ab	7.95a	0.21a	0.56ab	0.02b	3.50b	2.00b	2.83b
CS	10.47b	7.16a	0.06b	0.65a	0.01b	4.03b	1.62b	4.20a
CC	14.82ab	8.32 a	0.09b	0.72a	0.05b	3.56 b	2.19b	3.05ab
CSCC	16.52a	4.19b	0.11 b	0.32b	0.11a	7.48 a	3.67a	2.93ab

743

744 **Figure captions**

745 **Fig 1** Peak numbers in water, MeOH, and $CHCl_3$ extracts determined by
746 ESI-FT-ICR-MS. Letters denote significant differences within each solvent
747 according to ANOVA with Tukey's post-hoc test. Control, corn stover is
748 removed after corn harvested. CS, corn stover retention. CC, both corn stover
749 and cover crop are removed after corn harvested. CSCC, cover crop is removed
750 but corn stover remains after corn harvested.

751 **Fig 2** Percentages of SOM biochemical classes with AI >0.5 in water, MeOH, and
752 $CHCl_3$ extracts analyzed by FT-ICR-MS. Letters denote significant differences
753 within each solvent according to ANOVA with Tukey's post-hoc test.

754 **Fig 3** Pairwise comparisons showing the unique compounds of SOM in two contrasting
755 treatments extracted by water (a), MeOH (b), and $CHCl_3$ (c) using van Krevelen
756 diagrams. Colors indicate in which treatment that compound is unique to the
757 other. Subplots of a1, b1, c1 indicate the pairwise comparison between CS and
758 Control. Subplots of a2, b2, c2 indicate the pairwise comparison between CSCC
759 and CC. Subplots of a3, b3, c3 indicate the pairwise comparison between CC
760 and Control. Subplots of a4, b4, c4 indicate the pairwise comparison between
761 CSCC and CS.

762 **Fig 4** NMDS plot from the SOM chemical classes detected by FT-ICR-MS

763 **Fig 5** The stack column chart of the relative abundances of dominant bacterial phyla
764 derived from 16S rRNA gene sequencing

765 **Fig 6** Multivariate analysis of soil microbial community and SOM compounds using
766 redundancy analysis (RDA). Ordinations are based on Bray-Curtis, which
767 utilizes relative OTU abundance information of soil bacterial community. SOM
768 biochemical classes are fit to the ordinations.
769
770 **Fig S1** The relative abundances of SOM compounds sequentially extracted by (a)
771 water, (b) MeOH, and (c) $CHCl_3$ determined by FT-ICR-MS. Control, corn
772 stover is removed after corn harvested. CS, corn stover retention. CC, both corn
773 stover and cover crop are removed after corn harvested. CSCC, cover crop is
774 removed but corn stover remains after corn harvested.
775 **Fig S2** Venn diagrams comparing the unique and common peaks extracted by different
776 solvents (a) water (b) MeOH (c) $CHCl_3$. Control, corn stover is removed after
777 corn harvested. CS, corn stover retention. CC, both corn stover and cover crop are
778 removed after corn harvested. CSCC, cover crop is removed but corn stover
779 remains after corn harvested.
780 **Fig S3** Percentages of unique compounds extracted by (a) water (b) MeOH (c) $CHCl_3$
781 in pairwise comparisons derived from Fig 3. Control, corn stover is removed after
782 corn harvested. CS, corn stover retention. CC, both corn stover and cover crop are
783 removed after corn harvested. CSCC, cover crop is removed but corn stover
784 remains after corn harvested.

785 **Fig S4** NMDS plot of soil bacterial community at the OTU level in the different

786 treatments. Control, corn stover is removed after corn harvested. CS, corn stover

787 retention. CC, both corn stover and cover crop are removed after corn harvested.

788 CSCC, cover crop is removed but corn stover remains after corn harvested.

Fig 1

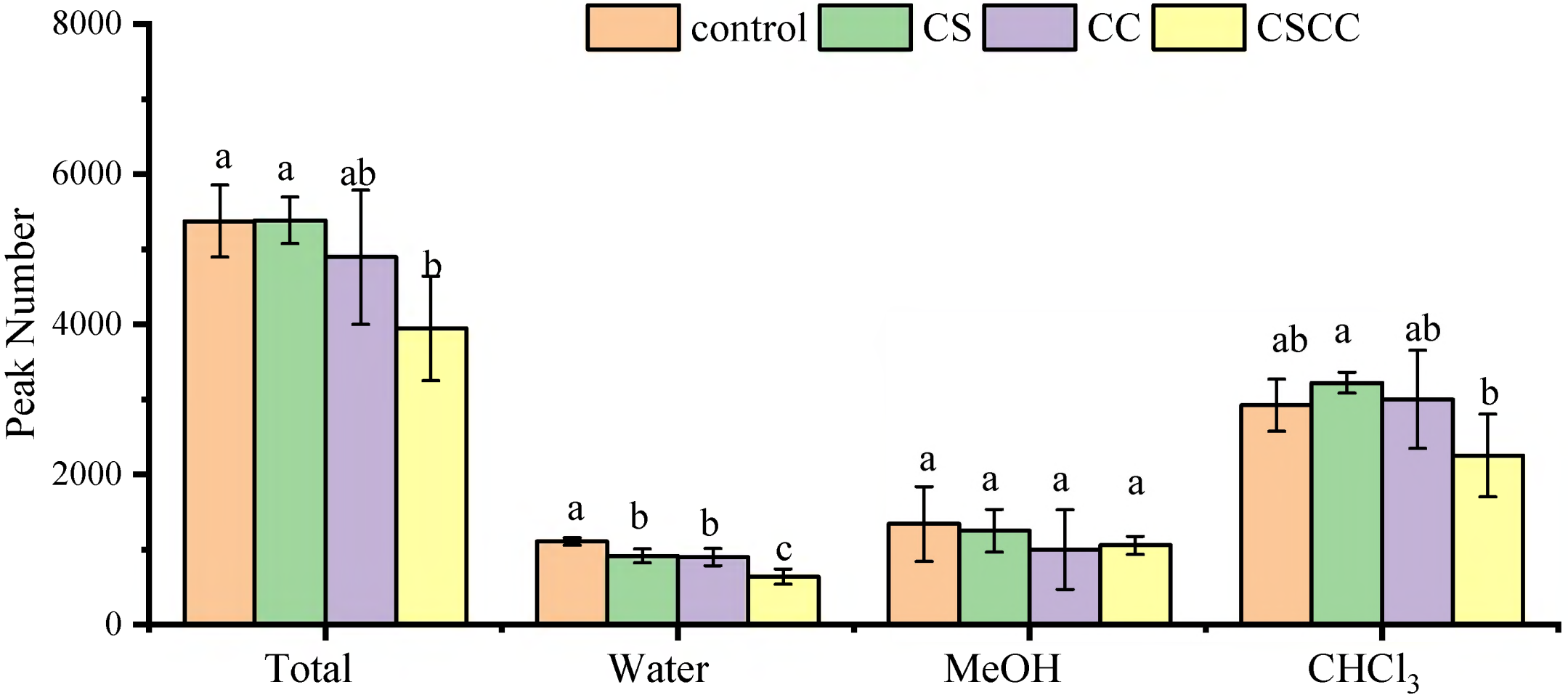

control
CS
CC
CSCC
Peak Number
8000
6000
4000
2000
0
a
a
ab
b
a
b
b
c
a
a
a
a
ab
a
ab
b
Total
Water
MeOH
CHCl3

Fig 2

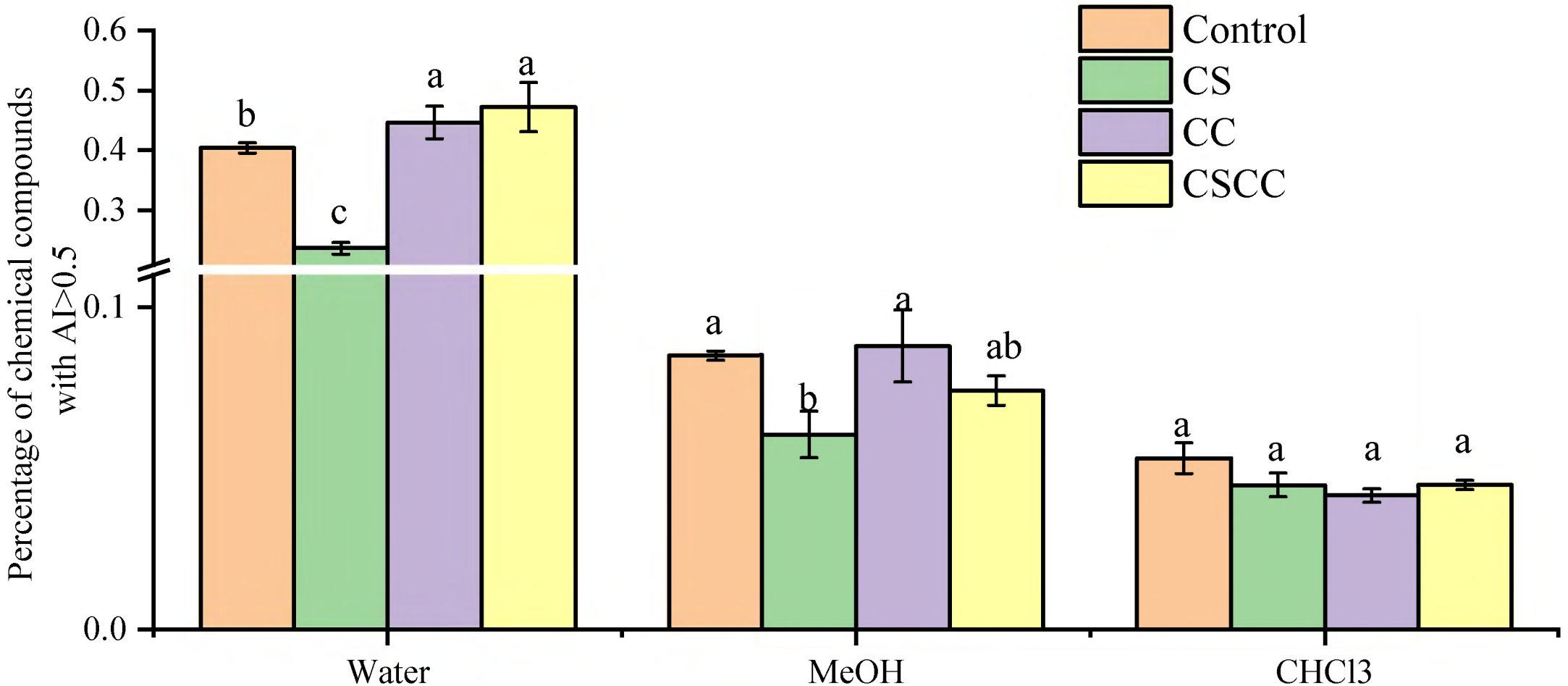

Control
CS
CC
CSCC
Percentage of chemical compounds with AI>0.5
0.6
0.5
0.4
0.3
0.1
0.0
b
c
a
a
a
b
a
ab
a
a
a
a
Water
MeOH
CHCl3

Fig 3

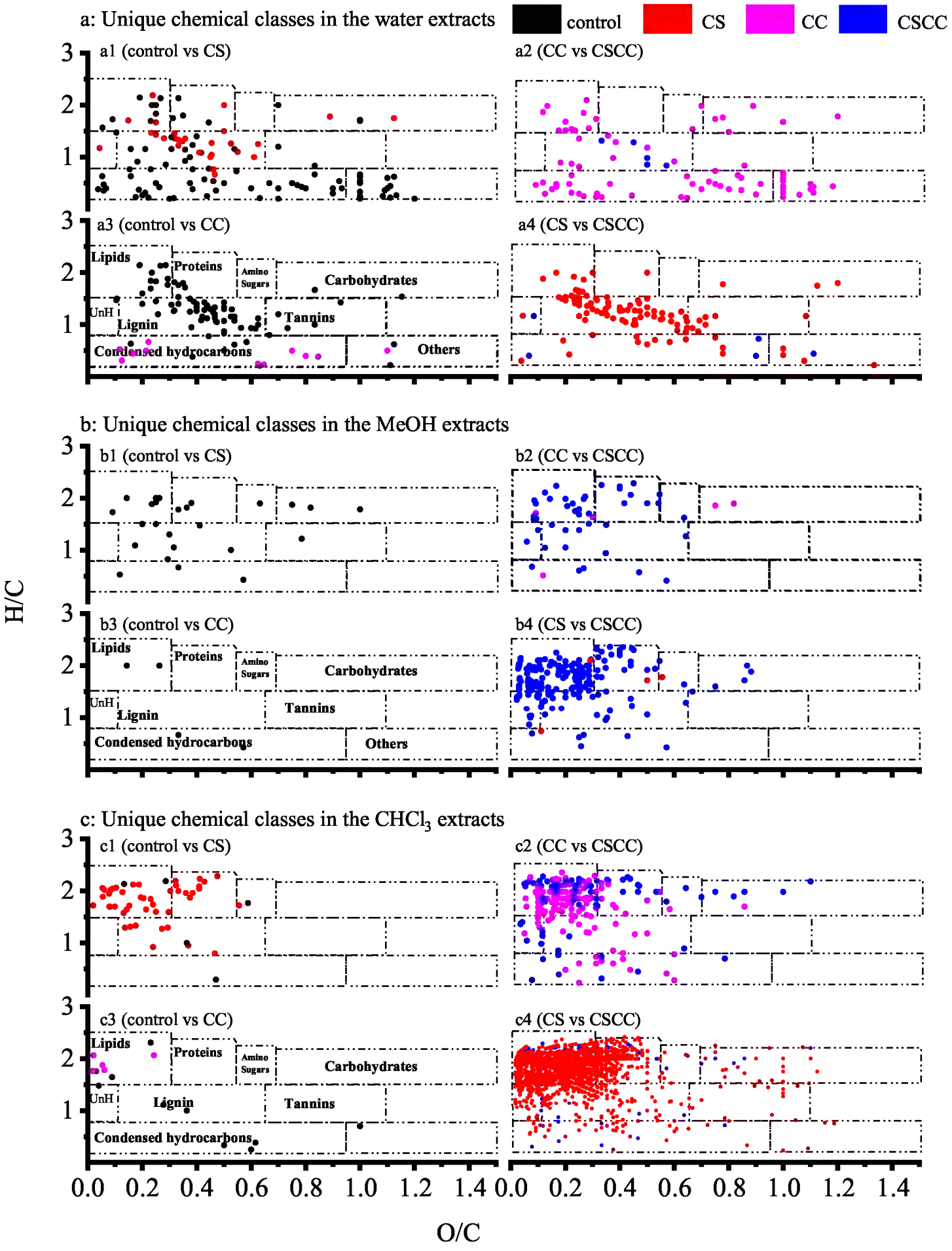

a: Unique chemical classes in the water extracts
control
CS
CC
CSCC
a1 (control vs CS)
a2 (CC vs CSCC)
a3 (control vs CC)
a4 (CS vs CSCC)
Lipids
Proteins
Amino Sugars
Carbohydrates
UnH
Lignin
Tannins
Condensed hydrocarbons
Others
b: Unique chemical classes in the MeOH extracts
b1 (control vs CS)
b2 (CC vs CSCC)
b3 (control vs CC)
b4 (CS vs CSCC)
c: Unique chemical classes in the CHCl3 extracts
c1 (control vs CS)
c2 (CC vs CSCC)
c3 (control vs CC)
c4 (CS vs CSCC)
H/C
O/C
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1
2
3

Fig 4

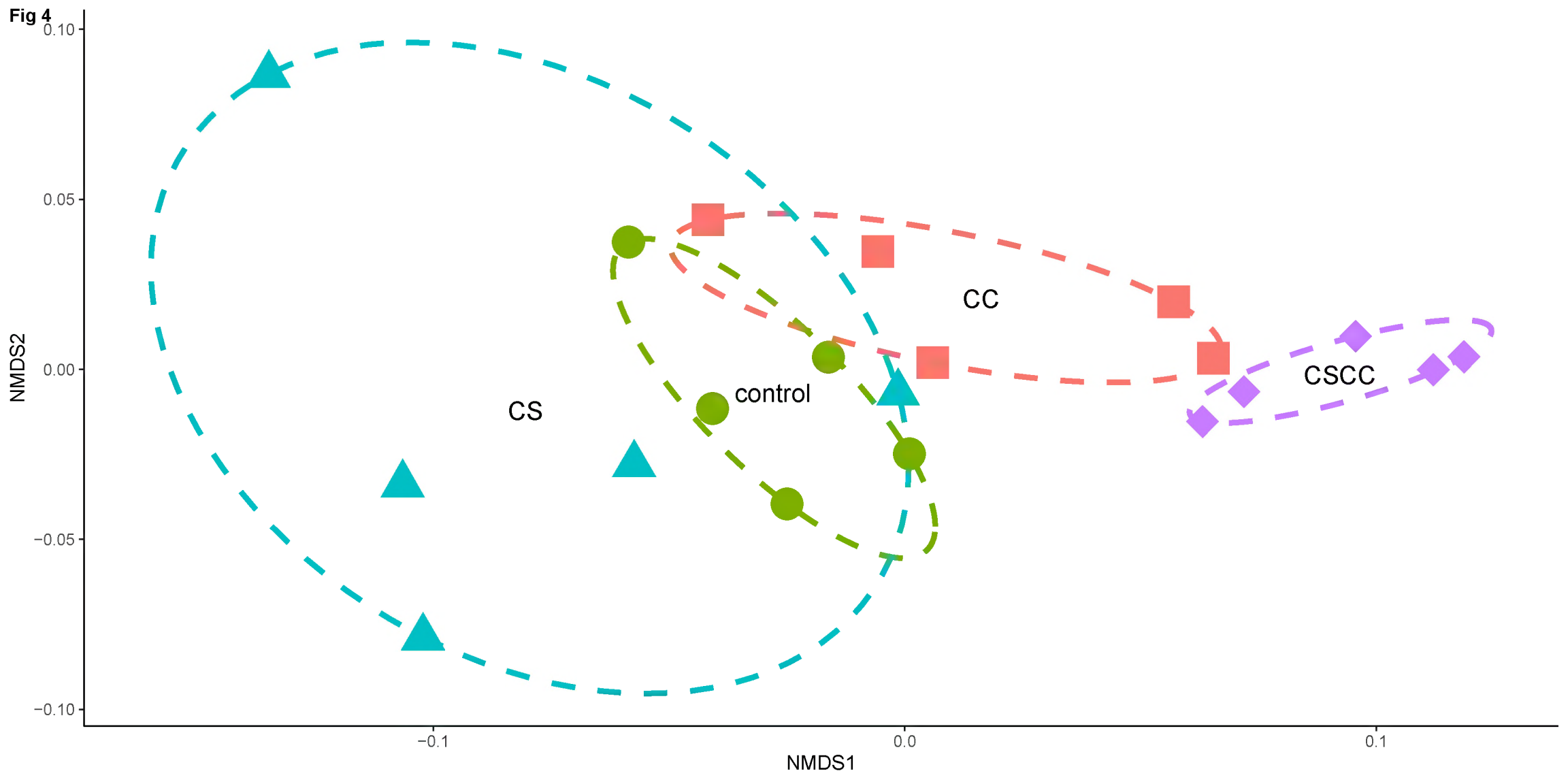


Fig 5

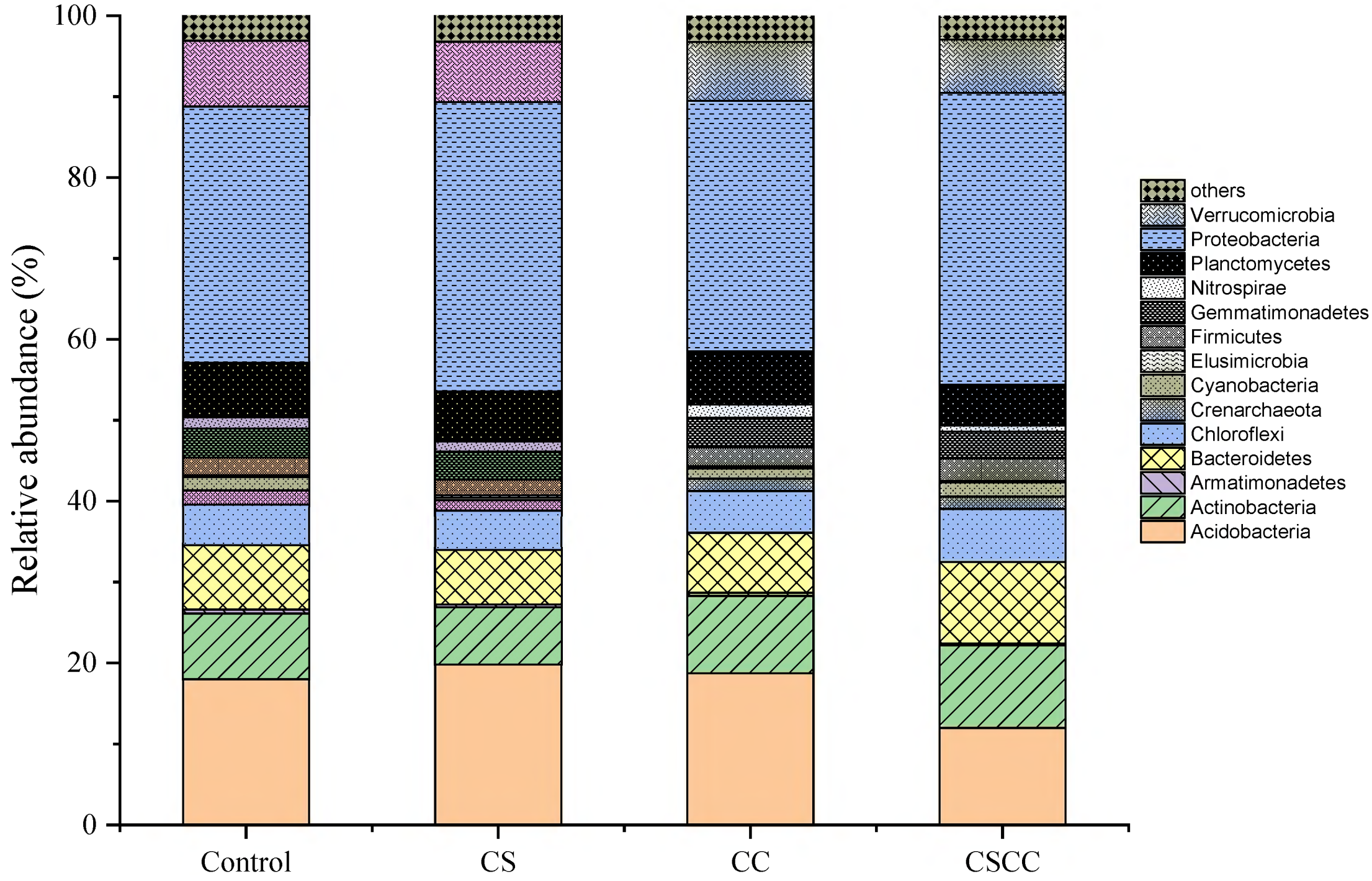
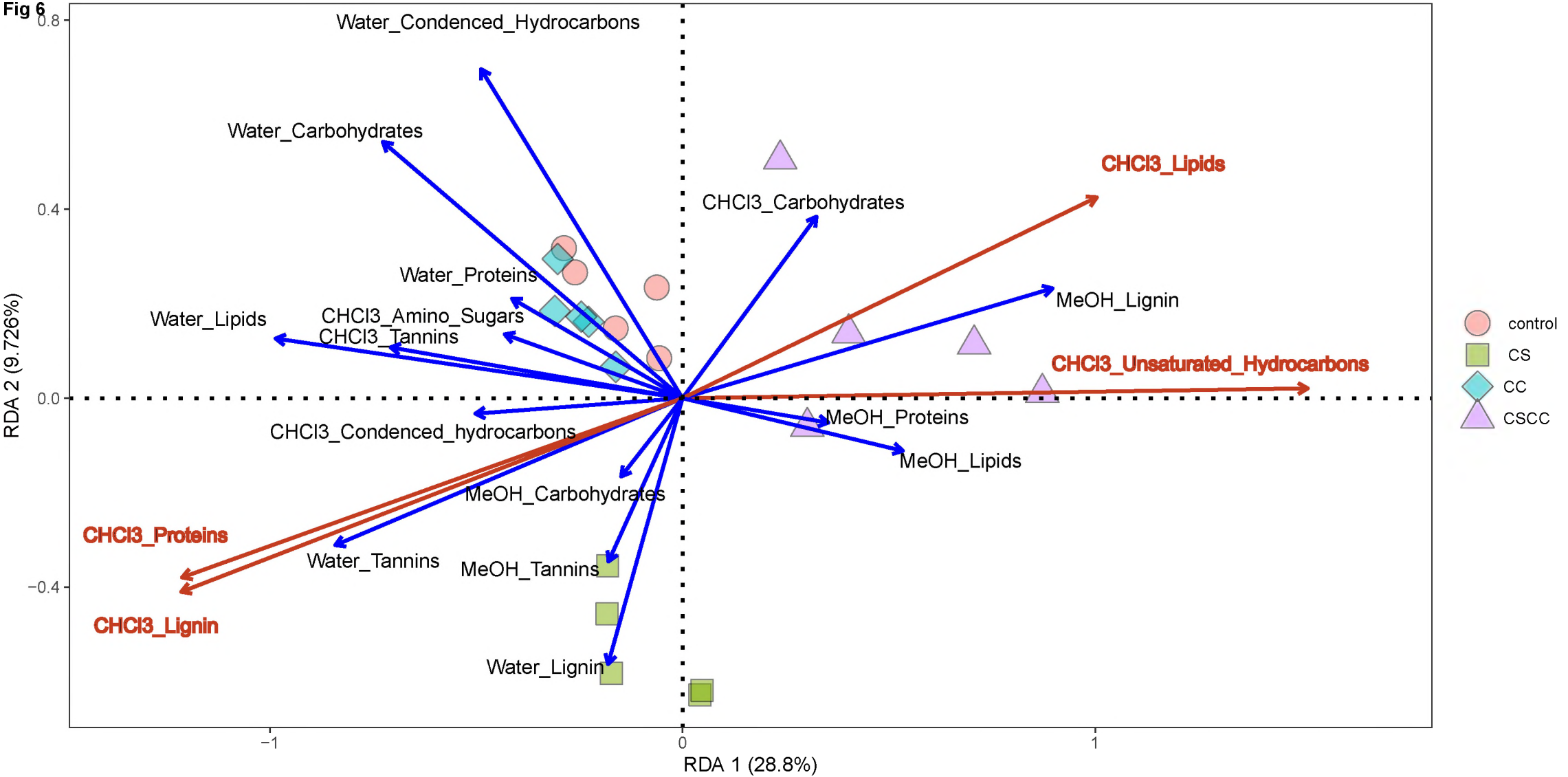

Relative abundance (%)
100
80
60
40
20
0
Control
CS
CC
CSCC
others
Verrucomicrobia
Proteobacteria
Planctomycetes
Nitrospirae
Gemmatimonadetes
Firmicutes
Elusimicrobia
Cyanobacteria
Crenarchaeota
Chloroflexi
Bacteroidetes
Armatimonadetes
Actinobacteria
Acidobacteria

Fig 6


Water_Condenced_Hydrocarbons
Water_Carbohydrates
CHCl3_Carbohydrates
CHCl3_Lipids
Water_Proteins
MeOH_Lignin
Water_Lipids
CHCl3_Amino_Sugars
CHCl3_Tannins
CHCl3_Unsaturated_Hydrocarbons
MeOH_Proteins
CHCl3_Condenced_hydrocarbons
MeOH_Lipids
MeOH_Carbohydrates
CHCl3_Proteins
Water_Tannins
MeOH_Tannins
CHCl3_Lignin
Water_Lignin
control
CS
CC
CSCC
RDA 2 (9.726%)
RDA 1 (28.8%)
0.8
0.4
0.0
−0.4
−1
0
1

*Declaration of Interest Statement

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: