1 **Title: Moderate N fertilizer reduction with straw return modulates ecosystem services and**

2 **microbial traits in a meadow soil**

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Abstract:

Nitrogen (N) fertilization has received worldwide attention due to its benefits to soil fertility and productivity, but excess N application also causes an array of ecosystem dis-services, such as greenhouse gas emissions. Soil microorganisms are considered to be involved in a variety of ecosystem services and dis-services. However, the relationships between soil ecosystem services and microbial traits under different N fertilizer application rates remain uncertain. To address this uncertainty, a 4-year in situ field experiment was conducted in a meadow soil on the Northeast China Plain. The experiment was performed after straw return with the following treatments, which included regular phosphorus (P) and potassium (K) fertilization: (i) regular N fertilizer (N+PK); (ii) 25% N fertilizer reduction (0.75N+PK); (iii) 50% N fertilizer reduction (0.5N+PK); and (IV) no N fertilizer (PK). Ecosystem services, dis-services and microbial traits responded distinctly to the different N fertilizer rates. The 0.75N+PK treatment had overall positive effects on soil fertility, productivity, straw decomposition, and microbial abundance and function and alleviated greenhouse gas emissions. Specifically, no significant differences were observed in SOC, total N and P contents, straw C and N release amounts, microbial biomass C and N contents, or cellulase and N-acetyl-D-glucosaminidase activities relative to those of 0.5N+PK and PK. Greenhouse gas mineralization was reduced with decreasing N input. Moreover, 0.75N+PK had the highest straw biomass and yield, which were significantly higher than those in 0.5N+PK and PK. Furthermore, 0.75N+PK increased aboveground biomass and soil C:N and increased the abundance of genes encoding cellulose-degrading enzymes, which implies the potential for C and N turnover. Most of the observed changes in ecosystem services and dis-services were strongly associated with specific microbial modules and keystone taxa. The *Lasiosphaeriaceae*-driven module 1 community promoted straw degradation and C and N release,

43   while the *Terrimonas*-driven module 3 community enhanced production, which was conducive to soil

44   multifunctionality. Therefore, our results suggest that straw return with a 25% reduction in chemical N

45   fertilizer is optimal for providing ecosystem services. This study highlights the importance of abiotic

46   and biotic factors in soil health and supports green agricultural development by establishing the optimal

47   N fertilizer rates in meadow soil after straw return.

48   **Keywords:** Ecosystem services; Straw return; Nitrogen fertilization; Microbial community; Crop yield

49   **1. Introduction**

50   Multiple soil ecosystem services are indicators of soil health (Kihara et al., 2020; Lehmann et al.,

51   2021). Soil ecosystem services are related to the ability of soil to function as a vital living system to

52   sustainably increase crop productivity, improve environmental quality, mitigate climate change and

53   promote plant and animal health (de Bello et al., 2010; Tang et al., 2019). Intensive agriculture has

54   posed a wide range of threats to agroecosystem services (Robertson et al., 2014; Allen et al., 2015).

55   Chemical fertilizers, especially nitrogen (N), have been inappropriately applied around the world to

56   increase crop yields in response to population growth (Shi et al., 2019). N is considered an essential

57   macronutrient for all biota, but excessive N fertilizer input not only reduces soil fertility and

58   productivity but also imposes environmental burdens (Trost et al., 2016). Recent research has indicated

59   that an appropriate reduction in N fertilizer input can not only maintain crop yield by increasing N

60   fertilizer use efficiency but also promote soil health by regulating the soil C:N ratio (Chen et al., 2014).

61   However, an excessive reduction in N fertilizer input can lead to an "N-mining" effect, resulting in the

62   loss of soil organic matter, which reduces crop yields (Chen et al., 2014). Therefore, how to maintain

63   agroecosystem services by regulating N fertilizer application rates needs to be fully assessed.

64   Straw return is widely implemented to support soil ecosystem services (Xu et al., 2021). Plant

65 residues contain abundant N, which affects soil fertility and productivity (Pan et al., 2009; Liu et al.,

66 2014). Thus, the N released during straw degradation is an important source that could partially

67 substitute chemical N fertilizer (Wang et al., 2017; Latifmanesh et al., 2020). However, crop fields with

68 abundant organic matter usually have low reutilization efficiency (Hou et al., 2020). Generally, most of

69 the N in straw is released into the atmosphere as oxynitrides, such as nitrous oxide (N_2O) (Wang et al.,

70 2019; Sun et al., 2021). Studies have shown that straw return significantly elevates greenhouse gas

71 emissions and that less than 15% of straw-derived N is converted into soil organic matter (SOM) (Yin

72 et al., 2018; Wu et al., 2019). However, whether straw can partially replace chemical N fertilizer is still

73 unclear.

74   Soil microorganisms are the drivers of soil ecosystem services (Handa et al., 2014; Wagg et al.,

75 2014). Agronomic management strategies targeting "multifunctionality" have prompted research into

76 the impact of microbes on achieving the desired rates of multiple ecosystem processes (Gong et al.,

77 2020). Fertilization-induced changes in microbial communities and functions regulate a variety of

78 ecosystem functions, including SOM formation, greenhouse gas emissions, litter decomposition, and

79 crop production (Dominati et al., 2014). For example, Ning et al. (2020) found that long-term manure

80 application increased the abundance of specific fungi involved in yield improvement. Duan et al. (2021)

81 reported that adequate N input improved the cellulose degradation ability of bacteria. Empirical

82 evidence of the relationships among N fertilizer application, specific microbial communities or

83 functions and multiple ecosystem services is still lacking, and the diverse cropland services driven by

84 complex microbial traits under different N fertilizer rates are unclear.

85   Microorganisms contribute to ecosystem services by modulating microbial function, community

86 composition and succession, which are influenced by N fertilizer input levels (Bradford et al., 2014;

87   Chen et al., 2019a). Generally, bacteria and fungi are the main drivers of the decomposition of labile

88   and recalcitrant compounds, respectively, in straw (Frey et al., 2013; Ge et al., 2017; Hogberg et al.,

89   2007). In addition, microbial modules and keystone taxa have been used to explain ecosystem services.

90   Chen et al. (2019b) found that specific microbial modules participated in N and phosphorus (P)

91   turnover in a Cambisol. Actinobacteria have been extensively studied and can be considered the main

92   degraders of straw due to their secretion of cellulase (Bao et al., 2021). C, N and P stoichiometry has

93   profound impacts on microbial in vivo metabolism and ex vivo modification processes (Chen et al.,

94   2016). Nevertheless, the understanding of the microbial mechanisms that modulate ecosystem services

95   in response to N fertilizer input levels is still limited.

96   As an important grain-producing region, the Northeast China Plain contributes more than 20% of

97   the total grain yield in China (Li et al., 2017; Zhao et al., 2018). Here, a field experiment was

98   conducted to reveal the influences of N input levels on soil ecosystem multifunctionality and associated

99   microbial traits. In the present study, two hypotheses were tested: (i) soil ecosystem services and

100   dis-services would show distinct responses to N fertilizer input levels, and (ii) the changes in cropland

101   ecosystem services and dis-services would be linked to specific microbial traits. The purpose of this

102   study was to optimize the N fertilizer application rate to achieve soil ecosystem multifunctionality and

103   explore the potential microbial mechanism in a Mollisol.

104   **2. Materials and methods**

105   **2.1 Site description and sampling**

106   A field experiment with varying inorganic N fertilizer input levels was established in 2018 in

107   Wenchun town (44°59′61″ N, 129°59′18″ E), Mudanjiang city, Heilongjiang Province, Northeast China

108   Plain, which is an important grain-producing area. This region has a typical temperate continental

109   monsoon climate with an average annual temperature of 4.3 °C and a mean annual precipitation of

110   579.7 mm. The soil is classified as a meadow soil according to the US Soil Taxonomy (USST). The

111   cropping system was a continuous maize (*Zea mays* L.) monoculture. Four treatments were established

112   with different N fertilizer input levels after straw return to the field for 4 years as follows: (1) regular

113   chemical fertilization, N+PK (300 kg urea (N 46%) ha^{-1} yr^{-1}, 250 kg diammonium phosphate (P$_2$O$_5$

114   48%) ha^{-1} yr^{-1}, 150 kg potassium chloride (K$_2$O 50%) ha^{-1} yr^{-1}); (2) 25% reduction of N fertilizer,

115   0.75N+PK (225 kg urea ha^{-1} yr^{-1}, 250 kg diammonium phosphate ha^{-1} yr^{-1}, 150 kg potassium chloride

116   ha^{-1} yr^{-1}); (3) 50% reduction of N fertilizer, 0.50N+PK (150 kg urea ha^{-1} yr^{-1}, 250 kg diammonium

117   phosphate ha^{-1} yr^{-1}, 150 kg potassium chloride ha^{-1} yr^{-1}); and (4) no N fertilizer, PK (250 kg

118   diammonium phosphate ha^{-1} yr^{-1}, 150 kg potassium chloride ha^{-1} yr^{-1}). All straw and chemical fertilizers

119   were applied with shallow tillage to 20 cm. Straw was cut into pieces less than 5 cm and returned to the

120   field after harvest in October, while the chemical fertilizers were applied during plowing in May of the

121   following year. All other normal management practices were consistent among the treatments during

122   the experiment. Before the experiment, the soil contained 18.74 g kg^{-1} SOC, 1.03 g kg^{-1} total N and

123   0.54 g kg^{-1} total P with a pH of 7.37 (H$_2$O). The yield and selected soil chemical properties under

124   different treatments during the experiment are shown in Supplemental material Table S1.

125   Soils were sampled after the maize harvest in October 2021. A randomized complete block design

126   consisting of 4 treatments with 3 replications was adopted. Each field plot was 4.5 m × 15 m. We

127   collected nine soil cores (5 cm diameter) from the top 20 cm of bulk soil in each plot. Each soil sample

128   consisted of a mixture of subsamples randomly collected at nine different positions in the same plot. In

129   total, 12 soil samples were collected from the 4 treatments. Each treatment included 3 replicates. Soils

130   were sieved through a 2 mm mesh, the mineral particles and plant roots were carefully removed, and

131   then the soils were homogenized and stored in an incubator at 4 °C in a 40% moisture environment.

132   One part of the bulk soil sample was air-dried to measure soil properties, and the other part was used

133   for microbial molecular analysis.

134   **2.2 Field straw decomposition and carbon and nitrogen release experiments**

135   The ditch-buried straw decomposition experiment was conducted using nylon litter bags. Maize

136   straw was collected after the maize harvest in 2020 and air-dried. Ten grams of maize straw was cut to

137   2 cm in length and put into nylon litter bags, which were then heat sealed. The bags were 6 cm × 10 cm

138   in size and were made of 200 mesh nylon fabric, which permitted the free transfer of microorganisms

139   between the nylon bags and soil. On May 2nd, 2021, litter bags containing straw were buried at 10 cm

140   depth in a random design to prevent bags associated with a given decomposition stage from being

141   placed together. The litter bags were collected after harvest on October 1st, 2021.

142   The straw decomposition ratio was calculated based on dry weight loss as (dry initial mass - dry

143   final mass)/dry initial mass. The straw C concentration was measured by titrimetry after oxidation with

144   a mixture of H_2SO_4 and $K_2Cr_2O_7$. Total N, P and K were determined using the Kjeldahl, molybdenum

145   blue colorimetry, and flame photometry methods, respectively. All methods have been described by Lu

146   (2000). The properties of the initial maize straw and collected maize straw samples are shown in

147   Supplemental material Table S2. The amounts of total straw C and N released were calculated by the

148   following equation:

149   Amount of total straw C or N released = (initial C (or N) content × dry initial mass - final C (or N)

150   content × dry final mass) × aboveground biomass

151   **2.3 Measurement of soil properties and assessment of ecosystem services**

152   Soil pH was measured at a soil:water ratio of 1:2.5 (weight/weight). Air-dried soil and 25 mL of

153   deionized water were shaken together for 1 min and left to settle for 30 min, and the soil pH was

154   determined using an electrode. Soil organic carbon (SOC) was measured by titrimetry after oxidation

155   with a mixture of H_2SO_4 and $K_2Cr_2O_7$. Total N and P were determined using the Kjeldahl and

156   molybdenum blue colorimetric methods, respectively. All of these methods have been described by Lu

157   (2000).

158   Microbial biomass C (MBC) and microbial biomass N (MBN) were analyzed using the

159   fumigation-extraction method. Ten grams of fresh soil was fumigated with chloroform in the dark for

160   24 h, and then the fumigated and nonfumigated soils were extracted with 0.5 M K_2SO_4 and shaken at

161   200 rpm for 0.5 h. Soil extracts were filtered through a 0.45-μm Millipore filter, and the C and N in the

162   extracts were determined using a Multi C/N 3100 analyzer (Analytik Jena AG). The C and N contents

163   in the nonfumigated soil extracts were subtracted from those in the fumigated soil extracts to determine

164   the C and N contents in the soil microbial biomass. Values of 0.45 and 0.54 were used to calibrate the

165   contents of MBC and MBN, respectively (Vance et al., 1987; Wu et al., 1990).

166   The activities of cellulase and N-acetyl-β-glucosaminidase were measured using

167   *p*-nitrophenyl-β-D-cellobioside and *p*-nitrophenyl-N-acetyl-β-D-glucosaminide as substrates,

168   respectively. Fresh soil (1.0 g) was mixed with 2.5 mL of 0.2 M acetate buffer (pH 5.0) and 2.5 mL of

169   0.02 M substrate and then shaken at 200 rpm and 37 °C for 1 h. The reaction was stopped by adding 1

170   mL of 0.5 M $CaCl_2$ and 4 mL of 0.1 M Tris buffer (pH 12.0). The mixture was vortexed and filtered,

171   and the concentration of *p*-nitrophenol (PNP) was measured by colorimetry at 400 nm. The same

172   procedure was followed for the controls, with the exception that the substrate was added after

173   incubation, and $CaCl_2$ and Tris buffer were added (Dick, 2011; Geisseler and Horwath, 2009).

174   To estimate the greenhouse gas emission potential, we conducted a 60-day incubation experiment.

175   Briefly, 20 g of fresh soil was placed in a 250-mL flask and then sealed with a gas-tight lid with a

176   rubber stopper in the middle. Gas samples (10 mL) were taken from the headspace of each flask at 1, 3,

177   7, 15, 30, and 60 days after sealing using a plastic syringe. Each gas sample was immediately injected

178   into a preevacuated 10-mL glass vial. The concentrations of methane (CH_4), N_2O and carbon dioxide

179   (CO_2) were determined using a gas chromatograph (Agilent 7890) with a flame ionization detector for

180   CO_2 and CH_4 and a ^{63}Ni electron capture detector for N_2O. The gas standards were provided by the

181   National Research Center for Certified Reference Materials, Beijing, China. The precision for

182   greenhouse gas concentrations was ±0.5% based on repeated measurements of gas standards (Qiu et al.,

183   2019). When the maize plants matured, all plants and grains were harvested from each plot, oven-dried

184   at 60 °C for 48 h and weighed. The straw aboveground biomass and crop yield were converted into

185   weight per hectare.

186   We selected 15 soil and crop properties to represent cropland ecosystem services; these properties

187   were related to the soil fertility index (SOC, total N, total P, MBC and MBN), greenhouse gas

188   emissions (CO_2, N_2O and CH_4), straw decomposition and C and N release, soil extracellular enzymes

189   (cellulase and N-acetyl-D-glucosaminidase), and maize biomass (aboveground biomass and crop yield).

190   Generally, SOC, total N and total P are the major soil fertility factors and indicate the nutrient status in

191   cropland, which can be related to soil fertility conditions. Microbial biomass reflects ecosystem

192   productivity. Greenhouse gas emissions are related to climate change and can be regulated by

193   fertilization regimes and soil microbial activities. Soil extracellular enzymes catalyze the

194   decomposition of a range of organic polymers, resulting in C and N turnover. Maize biomass

195   parameters (such as aboveground biomass and crop yield) reflect soil productivity. All of these

196   variables contribute to cropland functions. To evaluate the functions of the cropland ecosystem under

197   different fertilization conditions, we calculated an integrated soil ecosystem multifunctionality index

198   for further analysis. Notably, the opposite numbers of greenhouse gas emissions were used to evaluate

199   their negative effects. Due to the lack of a specific definition of multifunctionality, we first calculated

200   the Z scores of the 15 measured variables and then obtained a multifunctionality value for each plot by

201   averaging these 15 Z scores (Chen et al., 2019).

202   **2.4 DNA extraction and quantification of fungal ITS sequences, bacterial 16S rRNA genes and**

203   **genes encoding cellulose-degrading enzymes**

204   Total DNA was extracted from 0.5 g of freeze-dried soil by using a Fast DNA Spin Kit for Soil

205   (MPBio, USA) according to the manufacturer's instructions and then dissolved in 50 μl of Tris-EDTA

206   buffer. The quality of the extracted DNA was characterized by electrophoresis on 1% (wt/vol) agarose

207   gel. The quantity and quality of the DNA were checked using a Nanodrop spectrophotometer

208   (Nanodrop, PeqLab, Germany). The extracted DNA samples were stored at −80 °C before molecular

209   analysis.

210   Bacterial and fungal abundances were determined to reveal the changes in microbial community

211   composition. The abundances of bacteria and fungi were measured according to modified procedures

212   (Fierer and Jackson., 2005). We selected the primers *338F/518R (338F: CCTACGGGAGGCAGCAG;*

213   *518R: ATTACCGCGGCTGCTGG)* and *NSI1/58A2R (NSI1: GTAGTCATATGCTTGTCT; 58A2R:*

214   *CATTCCCCGTTACCCGTT)* for the qPCR assay. The thermal qPCR profiles for bacteria and fungi

215   were as follows: 95 °C for 2 min for DNA denaturation, 35 cycles of (95 °C for 30 s, 60 °C for 30 s,

216   72 °C for 30 s, and 80 °C for 15 s) for DNA annealing, and 81 °C for 10 s for DNA extension; and

217   95 °C for 10 min for DNA denaturation, 40 cycles of (95 °C for 15 s, 52 °C for 30 s, 72 °C for 30 s,

218   and 79 °C for 30 s) for DNA annealing, and 81 °C for 10 s for DNA extension, respectively. The initial

219 concentrations of the two plasmids used as standards for the bacterial and fungal abundance analyses

220 were 1.22×10^{10} and 9.05×10^{9}, respectively.

221   The fungal *cbhI* gene and bacterial *GH48* gene were selected as functional biomarkers of

222 cellulolytic fungi and bacteria, respectively. The primers *GH48 F8*/*GH48 R5* (*GH48_F8*: 5 -

223 GCCADGHTBGGCG ACTACCT - 3; *GH48_R5*: 5 - CGCCCCABGMSWWGTACCA - 3) and *cbhI*

224 *F*/*cbhI R* (*cbhI F*: ACCAAYTGCTAYACIRGYAA; *cbhI R*: GCYTCCCAIATRTCCATC) were used for

225 the qPCR assay. The abundances of bacterial *GH48* and fungal *cbhI* genes were quantified according to

226 modified procedures (Zhang et al., 2017). The qPCR thermal profiles for the target genes of *GH48* and

227 *cbhI* were as follows: 95 °C for 5 min for DNA denaturation, 40 × (94 °C for 30 s, 60 °C for 45 s, and

228 72 °C for 90 s) for DNA annealing, and 84 °C for 10 s for DNA extension; and 94 °C for 4 min for

229 DNA denaturation, 40 × (94 °C for 45 s, 50 °C for 30 s, and 72 °C for 60 s) for DNA annealing, and

230 81 °C for 10 s for DNA extension, respectively. The initial concentrations of the two plasmids used as

231 standards for bacterial *GH48* and fungal *cbhI* gene abundance analysis corresponded to 1.85×10^{11} and

232 2.65×10^{10} copies g^{-1} dry soil, respectively. qPCR was performed in triplicate, and amplification

233 efficiencies higher than 95% were obtained with r^2 values > 0.99.

234 **2.5 Bacterial 16S rRNA gene and fungal ITS amplification and sequencing**

235   High-throughput sequencing was performed with the Illumina MiSeq sequencing platform

236 (Illumina, Inc.). Both the forward and reverse primers were tagged with an adapter and linker sequence,

237 and 8-bp barcode oligonucleotides were added to distinguish the amplicons from different soil samples.

238   The primers *515F* *(5'-GTGCCAGCMGCCGCGGTAA-3')* and *907R*

239 *(5'-CCGTCAATTCMTTTRAGTTT-3')* were chosen to amplify the V4–V5 hypervariable region of the

240 16S rRNA gene. PCR was conducted in a 50-μL reaction mixture containing 27 μL of ddH$_2$O, 2 μL (5

241   µM) of each forward/reverse primer, 2.5 µL (10 ng) of template DNA, 5 µL (2.5 mM) of

242   deoxynucleoside triphosphates, 10 µL of 5× Fastpfu buffer, 0.5 µL of bovine serum albumin, and 1 µL

243   of TransStart Fastpfu polymerase (TransGen, Beijing, China). The PCR conditions were 94 °C for 5

244   min; 30 cycles of 94 °C for 30 s, 52 °C for 30 s and 72 °C for 30 s of extension; and 72 °C for 10 min

245   (Caporaso et al., 2010).

246   The fungal ITS1 region was amplified using the primer pair *ITS1F*

247   *(CTTGGTCATTTAGAGGAAGTAA)/ITS2 (GCTGCGTTCTTCATCGATGC)* (Ghannoum et al., 2010).

248   The 50-µL reaction mixture consisted of 1 µl (30 ng) of DNA, 4 µl (1 µM) of each forward/reverse

249   primer, 25 µl of PCR Master Mix, and 16 µl of ddH$_2$O. PCR amplification was conducted at 98 °C for

250   3 min, followed by 30 cycles (98 °C for 45 s, 55 °C for 45 s, and 72 °C for 45 s), with a final extension

251   at 72 °C for 7 min (Ghannoum et al., 2010). All amplicons were cleaned and pooled in equimolar

252   concentrations in a single tube, after which they were subjected to library preparation, cluster

253   generation, and 250-bp paired-end sequencing on the Illumina MiSeq platform (Illumina Inc., San

254   Diego, CA, USA).

255   The raw sequence data were processed using the Qualitative Insights into Microbial Ecology

256   (QIIME) pipeline (Caporaso et al., 2010). Sequences that fully matched the barcodes were selected and

257   distributed into separate files for the bacterial 16S rRNA and fungal ITS genes. Poor-quality sequences

258   with a length less than 200 bp (for fungal ITS) and 500 bp (for bacterial 16S) and a quality score less

259   than 20 were discarded, and chimeras were removed using the UCHIME algorithm (Edgar et al., 2010).

260   The remaining sequences were assigned to operational taxonomic units (OTUs) with a 97% similarity

261   threshold using UCLUST (Edgar, 2010). Alpha diversity and Bray–Curtis distances for principal

262   coordinate analysis of the soil microbial community were calculated after rarefying all samples to the

263   same sequencing depth.

264   **2.6 Statistical analysis**

265   The soil ecosystem multifunctionality index, crop yield, microbial traits and other relevant soil

266   variables among treatments were subjected to a chi-square test for independence of variance.

267   Significant differences were determined by one-way analysis of variance (ANOVA) based on the post

268   hoc Tukey test at the 5% level. Prior to ANOVA, the normality and homogeneity of variances were

269   tested by the Kolmogorov–Smirnov test and Levene's test, respectively. If normality was not met, log

270   or square-root transformation was implemented. One-way ANOVA was performed using SPSS 21.0

271   (SPSS Inc., Chicago, IL, USA).

272   Nonmetric multidimensional scaling (NMDS) analysis was used to evaluate the microbial

273   community composition. NMDS was performed in the "Vegan" package of R (4.0.2). Analysis of

274   similarities (ANOSIM) was used to examine the significant differences in microbial community

275   structure under different fertilization treatments. To describe the complex co-occurrence patterns of

276   various organisms, we constructed co-occurrence networks. We focused on the abundant microbial

277   phylotypes (with an average relative abundance > 0.01% for bacteria and fungi) for network

278   construction. Nodes with Pearson correlations greater than 0.70 and $p < 0.05$ were retained. Network

279   visualization of microbial taxa and ecological clusters of microbial phylotypes was conducted with

280   Gephi software. To obtain the keystone species of each network, a *Zi-Pi* plot series was constructed to

281   determine the role of each OTU. According to Deng et al. (2012), the plot includes (a) peripheral nodes

282   ($Z \leq 0.25$, $P \leq 0.62$), (b) module hubs ($Z > 0.25$, $P \leq 0.62$), (c) connectors ($Z \leq 0.25$, $P > 0.62$) and (d)

283   network hubs ($Z > 0.25$, $P > 0.62$). From an ecological perspective, OTUs in module hubs, connectors

284   and network hubs may be regarded as the microbial keystone taxa of the network systems (Deng et al.,

285   2015).

286   A first heatmap was constructed to reveal the associations between soil ecosystem services and

287   microbial module communities. Another heatmap was constructed to reveal the associations between

288   microbial traits and fertilizers, soil properties, greenhouse emissions and ecosystem multifunctionality.

289   The random forest algorithm was applied in the R package (4.0.2) "RandomForest" to estimate the

290   importance of the predictors of soil properties and microbial traits with respect to ecosystem

291   multifunctionality.

292   **3. Results**

293   **3.1 Cropland ecosystem services**

294   Data collection after the continuous 4-year in situ field experiment under different N input levels

295   revealed changes in cropland ecosystem services (Fig. 1). In terms of soil fertility, compared with those

296   in the N-limited treatments (PK and 0.5N+PK), the SOC and total P contents in the N+PK and

297   0.75N+PK treatments increased significantly (Fig. 1a, c) ($P < 0.05$), while there were no significant

298   changes in total N content (Fig. 1b). After straw decomposition (Fig. 1d), the amounts of straw C (Fig.

299   1e) and N (Fig. 1f) released showed different responses to varying N fertilizer input levels. Generally,

300   the N-rich treatments (N+PK and 0.75N+PK) significantly increased the straw decomposition rate and

301   straw C and N release relative to the N-limited treatments ($P < 0.05$). However, there was no

302   significant difference between N+PK and 0.75N+PK. The MBC and MBN contents, as well as the

303   associated enzyme activities, changed after the application of different N fertilizer rates (Fig. 1g, h, i

304   and j). The MBC (Fig. 1g) and MBN (Fig. 1h) contents were significantly higher in the N-rich

305   treatments than in the other treatments. However, the highest cellulase activity was observed in the

306   0.75N+PK treatment, which was significantly higher than that in the other treatments (Fig. 1i) ($P <$

307   0.05), and the N-acetyl-D-glucosaminidase activity decreased with decreasing N application (Fig. 1j).

308   Regarding greenhouse gas emissions, with decreasing N fertilizer application levels, CO_2 and N_2O

309   emissions gradually decreased (Fig. 1k, m). No significant difference in CH_4 emissions was detected

310   among the different fertilization treatments (Fig. 1l). In addition, the N fertilizer level had a strong

311   influence on maize yield and aboveground biomass (Fig. 1n, o). As expected, the 0.75N+PK treatment

312   achieved the highest multifunctionality index (0.61), followed by the N+PK (0.32), 0.5N+PK (-0.34)

313   and PK (-0.59) treatments (Fig. 1p).

314   However, although the 0.75N+PK treatment increased the straw N release amount and may have

315   met the requirements for plant growth, the total N input was still dominated by inorganic N input (Fig.

316   S1). Therefore, the N released from the straw could not offset the deficiency of N fertilizer.

317   Additionally, different N fertilizer input levels significantly changed the stoichiometry of C, N and P

318   (Fig. S2). Notably, the 0.75N+PK treatment significantly increased the C:N ratio compared with the

319   0.5N+PK and PK treatments ($P < 0.05$). The lowest C:N ratio was obtained for the 0.5N+PK treatment

320   (Fig. S2a). The N:P and C:P ratios showed no significant difference regardless of nutrient quantity (i.e.,

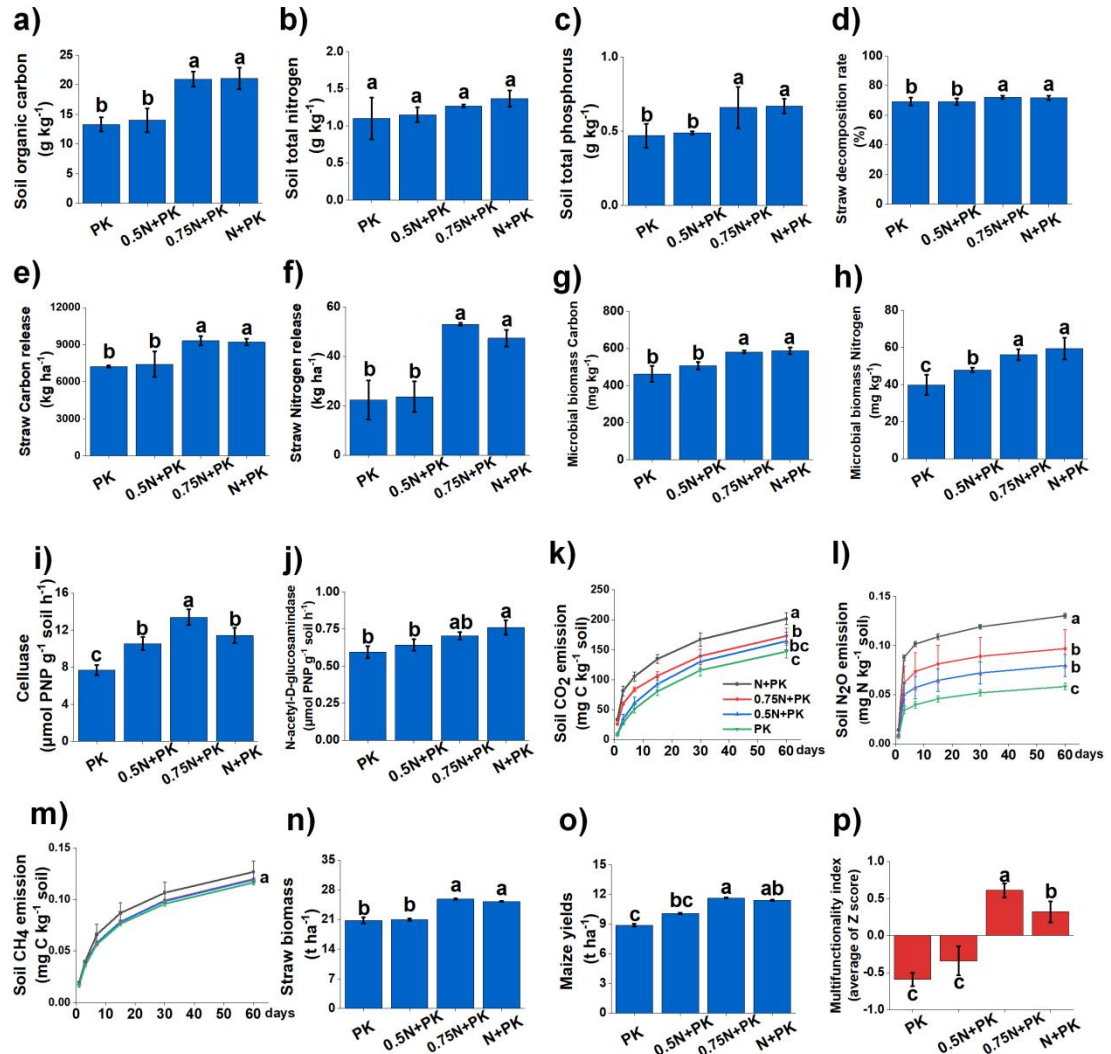
321   excess or limited) (Fig. S2b and c).

322

Fig. 1 Fifteen cropland variables and multifunctionality index values under different N input levels

after straw return. Abbreviations: N+PK, straw return plus regular inorganic N-P-K fertilizers;

0.75N+PK, straw return plus regular inorganic P-K with 25% N fertilizer reduction; 0.5N+PK, straw

return plus regular inorganic P-K with 50% N fertilizer reduction; PK, straw return plus regular

inorganic P-K without N fertilizer.

3.2 Abundances of bacteria, fungi and genes encoding cellulose-degrading enzymes

N fertilizer input levels had marked impacts on the abundances of fungi and bacteria (Table S3).

The highest fungal abundance was observed in the 0.75N+PK treatment, which was significantly

higher than that in the other treatments ($P < 0.05$). The N+PK treatment significantly increased

bacterial abundance compared with the PK treatment ($P < 0.05$), while there were no obvious

differences among the N+PK, 0.75N+PK and PK treatments. The ratio of fungi to bacteria also showed

contrasting responses to N fertilization (Table S3). The 0.75N+PK treatment significantly increased the

ratio of fungi to bacteria compared with the other treatments ($P < 0.05$), and the lowest ratio of fungi to

bacteria was found in the PK treatment.

Table 1 Abundances of genes encoding cellulose-degrading enzymes

across different N fertilizer treatments after straw return

Treatment	*cbhI* gene abundance (×10⁶ copies g⁻¹ soil)	*GH48* gene abundance (×10⁷ copies g⁻¹ soil)	*cbhI*: *GH48* ratio
N+PK	4.75±0.16 a	1.68±0.01 a	0.28±0.01 a
0.75N+PK	4.95±0.19 a	1.60±0.04 a	0.31±0.02 a
0.5N+PK	4.01±0.12 b	1.54±0.08 a	0.26±0.03 b
PK	3.76±0.13 b	1.40±0.06 b	0.27±0.02 b

The results are presented as the means ± standard deviations (n = 3). Different lowercase

letters after values indicate significant differences between each treatment, $P < 0.05$. N+PK,

straw return plus regular inorganic N-P-K fertilizers; 0.75N+PK, straw return plus regular

inorganic P-K with 25% N fertilizer reduction; 0.5N+PK, straw return plus regular inorganic

P-K with 50% N fertilizer reduction; PK, straw return plus regular inorganic P-K without N

fertilizer.

N fertilizer input levels led to changes in the expression levels of genes encoding

cellulose-degrading enzymes (Table 1). The N-rich treatments resulted in greater fungal *cbhI* and

bacterial *GH48* gene abundance than the N-limited treatments. In contrast, the highest *cbhI* gene

349   abundance was detected in the 0.75N+PK treatment, while the highest *GH48* gene abundance was

350   detected in the N+PK treatment. Compared with that in the PK treatment, the ratio of the fungal *cbhI*

351   gene to the bacterial *GH48* gene increased significantly in the 0.75N+PK treatment ($P < 0.05$).

3.3 Co-occurrence network analysis of the microbial community

353   Regarding fungal alpha diversity, there were no significant differences in the Chao1 index across

354   treatments. The N+PK treatment significantly increased fungal richness compared with the PK

355   treatment ($P < 0.05$) (Table S4). In addition, the PK treatment resulted in lower bacterial richness than

356   the other treatments ($P < 0.05$). No significant difference was observed in the bacterial Chao1 index

357   across treatments (Table S4). The NMDS plots showed that different N input levels significantly

358   changed the fungal (Fig. S3a) and bacterial communities (Fig. S3b) ($P < 0.05$).

359   We further conducted network analysis to identify co-occurrence patterns between specific

360   microbial taxa (Fig. 2). The cooccurrence network was aggregated into smaller coherent modules that

361   were examined to determine important module–trait relationships. The network comprised 1963 nodes

362   (composed of 1520 bacterial taxa and 443 fungal taxa) and 62206 edges with 52.49% positive

363   associations (Fig. 2a). The results showed that four dominant ecological modules (1-4) strongly

364   co-occurred within the multitrophic network and contributed 86.10% of the whole network. Among the

365   four modules, bacteria accounted for the highest proportion in each module, contributing more than

366   70% of the total (Fig. 2b). The percentage of edges linking bacteria to bacteria (B-B) was higher than

367   that of edges linking fungi to fungi (F-F) or bacteria to fungi (B-F). The highest proportion of B-B

368   (80.32%) was found in Module 3, while the highest proportions of B-F (32.66%) and F-F (6.00%) were

369   found in Module 4 (Fig. 2c).

370

Fig. 2 Relationships of microbial module communities with soil ecosystem services and

dis-services. Multitrophic network including multiple ecological modules. The colors of the nodes

represent different ecological modules (a). OTU number proportions of bacteria and fungi (b).

Proportions of edges linking bacteria to bacteria (B-B), bacteria to fungi (B-F) and fungi to fungi

(F-F) in the major ecological modules (c). Links connecting specific module communities with

soil ecosystem services and dis-services (d). * indicates significance at $P < 0.05$. Abbreviations:

SOC, soil organic carbon; C:N, ratio of SOC content to total N content; N:P, ratio of total N

380   content to total P content.

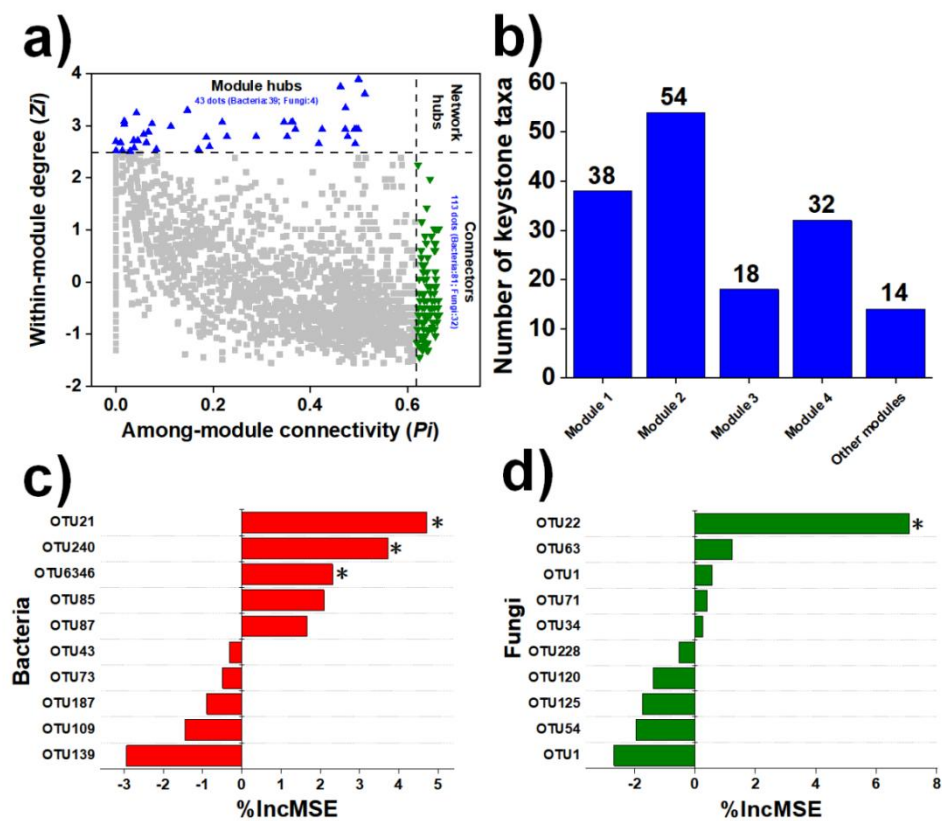
381   Individual nodes represent different roles in the microbial network based on the intramodule

382   connectivity Zi and the intermodule connectivity Pi. ZP plots were constructed to identify the

383   topological roles of each node in the network (Fig. 3a). As shown in Fig. 3b, 113 microbial taxa (81

384   bacterial species and 32 fungal species) were regarded as connectors, and 43 microbial taxa (39

385   bacterial species and 4 fungal species) were regarded as module hubs. Specifically, module 2 (54)

386   contained the most keystone taxa, followed by module 1 (38) and module 3 (32).

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388   **Fig. 3** Topological roles of microbial taxa and their effects on the soil multifunctionality index.

389   The topological role of each OTU was determined according to the scatter plot of within-module

390   connectivity (Z) and among-module connectivity (P) (a). Distribution of keystone taxa in each

391   ecological module (b). Contributions of bacterial (c) and fungal OTUs (d) to the soil

392   multifunctionality index. *, ** and *** indicate significance at $P < 0.05$, 0.01 and 0.001,

393   respectively.

3.4 Relationships between microbial traits and soil ecosystem multifunctionality

395   The heatmap showed close correlations of fertilizers (N input and straw return) with soil

396   stoichiometry and microbial traits (Fig. 4). Overall, the N input level, straw biomass and C:N ratio

397   increased the abundance of genes encoding cellulose-degrading enzymes. In addition, N input was

398   positively correlated with bacterial abundance, and a significant correlation was observed between

399   straw biomass and the N input level. The random forest model was used to identify abiotic and biotic

400   attributes correlated with soil ecosystem multifunctionality (Fig. 5). The model explained 83.89% of

401   the variance in ecosystem multifunctionality. The results indicated that the N input level, straw biomass

402   and soil C:N ratio were the predominant abiotic factors affecting the ecosystem multifunctionality

403   index; some biotic factors, such as the abundance of genes encoding cellulose-degrading enzymes,

404   significantly affected the ecosystem multifunctionality index.

405   Moreover, to clarify the potential main drivers of soil ecosystem services, the correlations between

406   microbial physiological traits and soil properties were calculated to determine the role of the microbial

407   community in soil ecosystem multifunctionality (Fig. 2d). The results indicated that the microbial

408   module community was significantly correlated with soil ecosystem services. The communities of

409   modules 1 and 2 and the fungal community in module 4 showed potential contributions to soil

410   ecosystem services (Fig. 2d). Specifically, significant correlations were observed between SOC content,

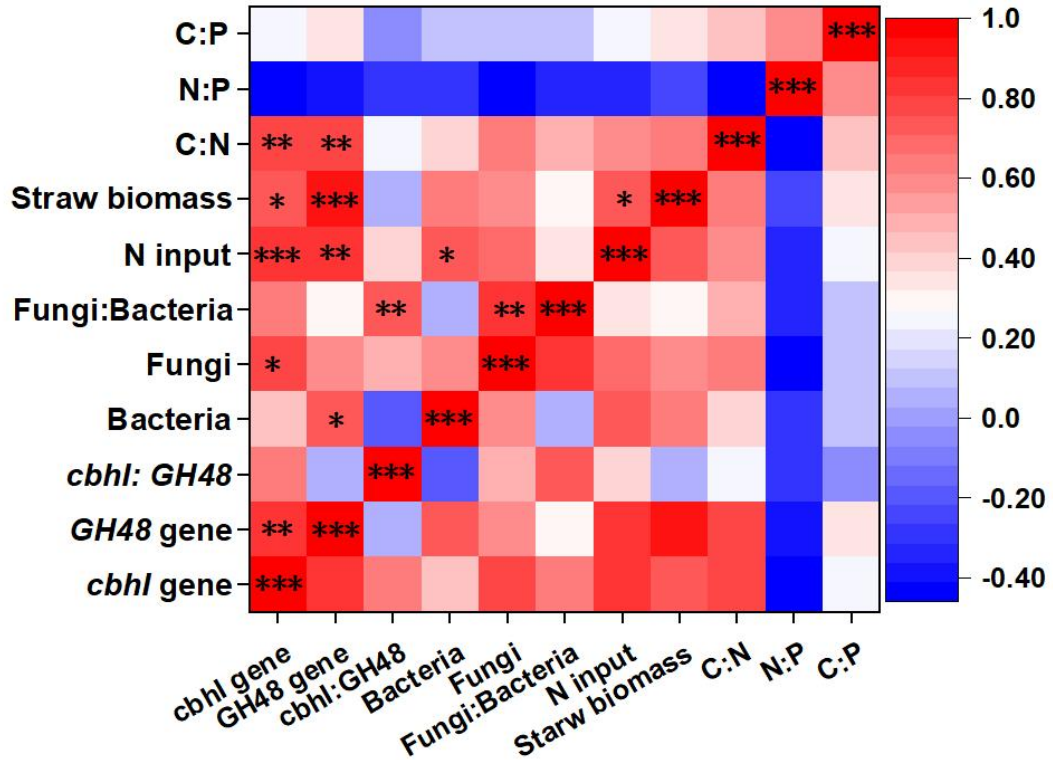
411   straw decomposition, straw C/N release, CO_2/N_2O mineralization and the module 1 community; the

412   module 2 community was positively correlated with greenhouse gas (except for CH_4) emissions; and

413   the fungal community in module 4 was positively correlated with SOC content, straw decomposition,

414   straw C/N release and straw biomass. Furthermore, the bacterial and fungal communities belonging to

415   module 2 and the fungal community belonging to module 3 were significantly correlated with CO_2

416   emission, maize yield and straw biomass.

417

418   **Fig. 4** Heatmap showing the close correlations of fertilizer (N input and straw return) and soil

419   stoichiometry with microbial traits. *, ** and *** indicate significance at $P < 0.05$, 0.01 and 0.001,

420   respectively. Abbreviations: C:N, ratio of SOC content to total N content; N:P, ratio of total N

421   content to total P content.

422   We selected the 20 keystone taxa at the species level (10 bacterial and 10 fungal taxa) with the

423   highest relative abundance for further analysis. The random forest model results indicated that specific

424   keystone taxa strongly influenced soil ecosystem multifunctionality (Fig. 3c and d). Bacterial OTU21

425   (in module 1), OTU240 (in module 2) and OTU6346 (in module 3) were highlighted as essential

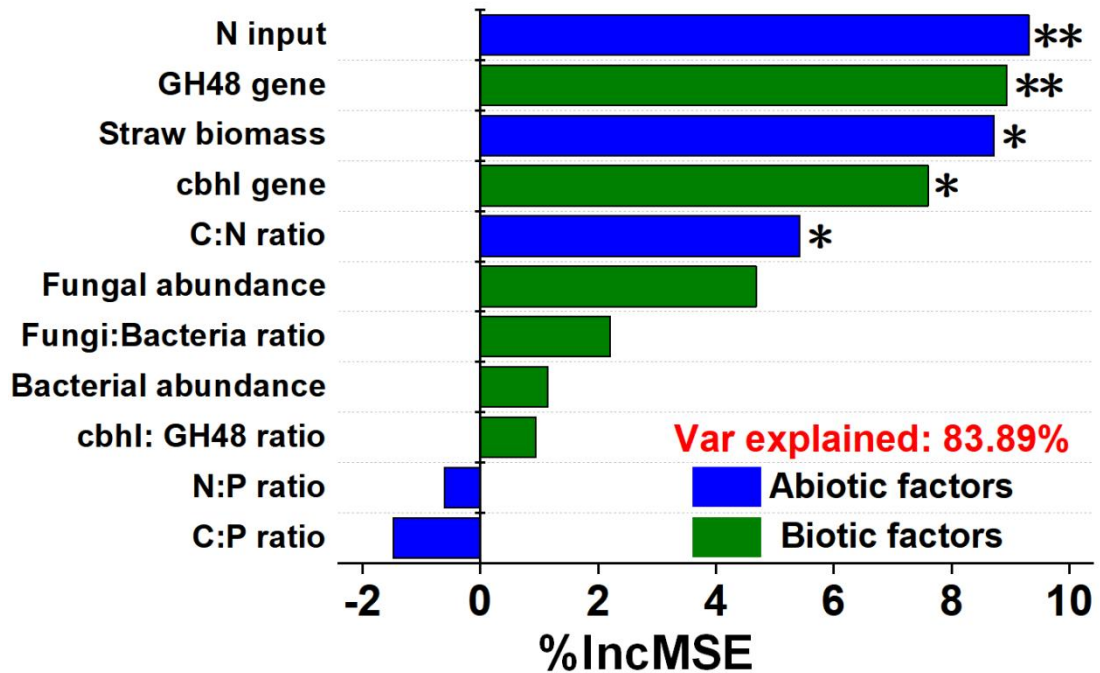
426   predictors of soil ecosystem multifunctionality, and fungal OTU22 (module 3) was also found to be an

427   important predictor. The relative abundances of selected keystone taxa differed among the different N

428   fertilizer treatments after straw return (Table S5). The relative abundances of fungal OTU22 and

429   bacterial OTU21 were higher in the N-rich treatments than in the N-limited treatments. Moreover,

430   compared with the N+PK treatment, the 0.75N+PK treatment increased the relative abundance of

431   fungal OTU22 by 38.20% and that of bacterial OTU21 by 40.63%.

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433   **Fig. 5** Contribution of abiotic and biotic variables to the soil multifunctionality index. *, ** and

434   *** indicate significance at $P < 0.05$, 0.01 and 0.001, respectively. Abbreviations: C:N, ratio of

435   SOC content to total N content; N:P, ratio of total N content to total P content.

436   **4. Discussion**

437   **4.1 Effect of N fertilizer reduction on cropland ecosystem services after straw return**

438   Soil fertility, straw decomposition, C and N release amounts, and crop productivity were mostly

439   higher under 0.75N+PK and N+PK than under the other treatments, implying that better soil

440   multifunctionality was achieved. Moreover, N+PK increased greenhouse gas emissions (Fig. 1). Higher

441   MBC and MBN values, as well as relevant enzyme activities, were also observed under the N-rich

442 treatments, indicating the strong positive impact of abundant N fertilizer application (Fig. 1g, h, i, j). It

443 has been reported that straw return with N fertilizer application can stimulate microbial activity and

444 promote biomass accumulation (Treseder, 2008). The substantial increases in straw decomposition and

445 straw C and N release under the N-rich treatments may be primarily attributed to microbial activity

446 (Fig. 1d, e, f), which is consistent with previous research (Ramirez et al., 2012). Our results indicated

447 that 0.75N+PK maintained parameters related to the soil fertility index and net primary production

448 compared to N+PK. The results demonstrate that the effects of 0.75N+PK on soil ecosystem services

449 are similar to those of N+PK. Therefore, it can be concluded that 0.75N+PK is a more efficient and

450 effective option for improving soil ecosystem services. Moreover, 0.75N+PK may enhance N fertilizer

451 use efficiency and stimulate microbial functioning by altering the stoichiometry of C, N and P in the

452 soil, ultimately promoting soil fertility and crop yield (Liu et al., 2010). Reducing the amount of N

453 fertilizer by more than 50% led to insufficient N input to meet the needs of both crops and microbes,

454 resulting in a decline in soil health (Williams et al., 2013). Recent studies have also proven that rational

455 N input can stimulate microbial ex vivo production of extracellular enzymes to accelerate straw

456 decomposition and nutrient transformation (Chen et al., 2016). Moreover, fungi have high nutrient

457 utilization efficiency; thus, more straw-derived C and N are stored in soil under N-rich treatments than

458 under N-limited treatments (Hou et al., 2020). Sufficient available N is a prerequisite for straw

459 decomposition and SOM formation according to the microbial "stoichiometric decomposition" theory,

460 while the "N-mining" theory, which applies to N-limited treatments, proposes that oligotrophic species

461 (such as K-strategists) degrade native SOM because of insufficient N input (Chen et al., 2014). In this

462 study, a 25% reduction in N fertilizer application may be the threshold value between N mining and

463 stoichiometric decomposition regimes. Finally, increases in SOC, total N and P contents and straw C

464   and N release, as well as microbial biomass and function, are commonly implicated in high

465   aboveground biomass and maize yields (Fig. 1n, o), which are favorable from the viewpoint of

466   ecosystem services.

467   However, excess N input also causes increased greenhouse gas emissions (Tang et al., 2019). In

468   the present study, greenhouse gas emissions were quantified to evaluate the ecosystem dis-services

469   under different N fertilizer input levels: the greater the emissions were, the lower the soil ecosystem

470   multifunctionality was (Fig. 1k, l, m). Straw return with N fertilizer addition might be a crucial driver

471   of CO_2 and N_2O emissions from agroecosystems and has been widely studied in the literature

472   (Gregorich et al., 2005). CO_2 and N_2O emissions increased significantly compared with those under the

473   PK treatment, likely due to stimulation of the activity of copiotrophs when sufficient C and N

474   substrates were provided. For example, on the basis of meta-analysis and field experiments, Dieleman

475   et al. (2010) found that CO_2 and N_2O increase as N input increases. Qiu et al. (2019) reported that CO_2

476   emissions enhanced root and mycorrhizal N uptake and increased N_2O emissions, which was related to

477   changes in soil denitrifier community composition favoring N_2O-producing taxa (nirK- or nirS-type).

478   In addition, there was no difference in CH_4 emissions among treatments, although contradictory results

479   have been widely reported in the literature (Tang et al., 2019). Mapanda et al. (2011) and Liu et al.

480   (2012) indicated that CH_4 emissions depend strongly on the soil water content in maize cropland,

481   which is consistent with our results. In summary, previous studies clearly demonstrated a positive

482   correlation between CO_2 emissions, N_2O emissions and N input. Accordingly, this study unequivocally

483   showed that N+PK emits more greenhouse gases than 0.75N+PK.

484   Overall, compared with the N+PK treatment, the 0.75N+PK treatment supported multiple

485   ecosystem services, including promotion of soil fertility, straw nutrient release and microbial activity

486 and mitigation of greenhouse gas emissions (Fig. 1p). Therefore, a 25% reduction in chemical N

487 fertilizer input with straw return may be an appropriate regime for promoting ecosystem services in

488 meadow soils on the Northeast China Plain.

489 **4.2 Responses of microbial abundance and function to straw return with N fertilizer reduction**

490 Fungal and bacterial abundances, as well as the ratio of fungi to bacteria, were sensitive to

491 changes in the N fertilizer input levels (Table S3 and Fig. 2). Straw addition with N fertilizer input

492 supplied enough C and N for microbial metabolism, thus promoting microbial proliferation (Chen et al.,

493 2016). Generally, bacterial abundance decreased with reduced N fertilizer input. This is mainly because

494 bacteria are more sensitive than fungi to N availability, which is consistent with the findings of a

495 previous study (Ramirez et al., 2020). Notably, compared with regular N input, a 25% reduction in N

496 fertilizer significantly increased fungal abundance. This result might be attributed to the negative effect

497 of excess N fertilizer (Wan et al., 2015). Moreover, Ning et al. (2020) performed 7 long-term field

498 experiments under different fertilization conditions across China and demonstrated that the C:N ratio

499 was a pivotal factor in fungal community composition; they also reported a significant positive

500 correlation between C:N ratio and fungal community composition. Gao et al. (2015) indicated that the

501 optimal ratio of C to N inputs was 20:1, which may meet the demands of maize growth and microbial

502 proliferation. It is well known that fungi have a stronger C utilization efficiency than bacteria (Duan et

503 al., 2021). Therefore, increasing fungal abundance and lowering the ratio of bacteria to fungi are

504 crucial for straw degradation and SOC accumulation. Previous studies have shown that the C:N ratio of

505 fungi is greater than 20; however, the C:N ratio of bacteria is less than 10. Excessive N fertilizer input

506 may reduce the soil C:N ratio, while low N fertilizer input cannot meet the growth requirements of

507 crops and microorganisms (Ning et al., 2020). Therefore, appropriate enhancement of the soil C:N ratio

508   can increase the ratio of fungi to bacteria, stimulate fungal function, and promote straw degradation and

509   SOC accumulation. Therefore, the 0.75N+PK treatment with a higher C:N ratio (16.47) may facilitate

510   the proliferation of microorganisms and promote an increase in microbial abundance.

511   Our results showed that the N-rich treatments resulted in higher microbial cellulose-degrading

512   gene abundances than the PK treatment (Table 1), which demonstrated the crucial role of N input in

513   straw degradation (Zhang et al., 2017). Additionally, the increase in fungal *cbhI* gene abundance

514   compared with bacterial *GH48* gene abundance required adequate N fertilizer input and was regulated

515   by the soil C:N ratio, which suggests that rational N fertilizer input could promote fungal degradation

516   of recalcitrant straw components (Hou et al., 2020). Therefore, the ratio of *cbhI* gene abundance to

517   *GH48* gene abundance was higher under the 0.75N+PK treatment than under the N-limited treatments

518   since the increased expression of a fungal cellulose-degrading gene implies increased straw C and N

519   release.

520   Our results indicated that 75%-100% N fertilizer could upregulate fungal and *cbhI* gene

521   abundances, which may lead to straw decomposition and SOC accumulation. It is therefore necessary

522   to further explore the potential associations between microbial traits and ecosystem services under

523   varying N fertilizer input levels.

524   **4.3 Relationships of cropland ecosystem services with microbial traits**

525   To clarify the effects of abiotic and biotic factors on soil ecosystem services, we quantified the

526   contributions of abiotic and biotic factors to the ecosystem multifunctionality index across the different

527   N input treatments (Figs. 4 and 5). Biotic factors, such as *cbhI* and *GH48* gene abundances, as well as

528   abiotic factors, including the C:N ratio, straw biomass and N input level, are also key regulators of

529   ecosystem multifunctionality (Fig. 5). In general, rapid straw degradation is an important way to

530 convert straw-C into SOM, thus improving soil fertility, aboveground biomass and crop yield. In

531 addition, fungi have a higher C utilization efficiency than bacteria; thus, a high fungal *cbhI* gene

532 abundance may correspond to better soil multifunctionality (Hou et al., 2020). Among abiotic factors,

533 the soil C:N ratio, straw biomass and N fertilizer input are regarded as the main indicators of soil

534 fertility and health, likely because they provide available nutrients and influence microbial community

535 composition (Ning et al., 2020).

536 Numerous studies have shown that core microbiota play a vital role in maintaining the stability of

537 soil microbial function and the complexity of microbial networks and in promoting ecosystem services

538 related to soil nutrient cycling (Ghannoum et al., 2015); moreover, keystone species may show great

539 explanatory power in terms of the structure and function of specific networks (or modules) (Chen et al.,

540 2019b). In the present study, *Terrimonas* (a bacterial species in module 1) and *Lasiosphaeriaceae* (a

541 fungal species in module 3) were identified as the keystone taxa influencing soil multifunctionality in

542 the co-occurrence network (Table S5). A previous study demonstrated that straw addition significantly

543 increased the relative abundance of *Lasiosphaeriaceae*, which implied the potential for straw

544 decomposition (Song et al., 2020). Later, *Lasiosphaeriaceae* was proven to promote straw-derived C

545 and N accumulation by secreting multiple extracellular enzymes (Guo et al., 2022). Moreover, Sun et al.

546 (2023) revealed that *Lasiosphaeriaceae* abundance was regulated by the soil C:N ratio and was

547 especially affected by changes in mineral N. Therefore, *Lasiosphaeriaceae* can effectively promote

548 straw degradation and straw C and N release while driving the function and community of module 1,

549 which is consistent with our results (Fig. 2d). However, relatively few studies have focused on the

550 function of *Terrimonas*, so this study focused on *Chitinophagaceae*. In the literature, straw return was

551 the main contributor to increases in *Chitinophagaceae* abundance (Li et al., 2021). Furthermore,

552 *Chitinophagaceae* was indicated to have a strong ability to accumulate soil C and N and degrade

553 cellulose (Zhong et al., 2022), thus promoting production by regulating the module 3 community and

554 function, which is in line with our results (Fig. 2d).

555   Overall, straw return with sufficient N fertilizer application can increase the C:N ratio and

556 stimulate microbial traits, which ultimately promote soil ecosystem multifunctionality (Fig. 6). Straw

557 return without a sufficient N supply cannot support ecosystem services due to the decomposition of

558 native SOM and the imbalanced microbial community composition, according to the N-mining theory

559 (Chen et al., 2014); straw return with sufficient N application (N+PK and 0.75N+PK) can promote soil

560 fertility, straw release, microbial activity and crop productivity, which can be explained by the

561 stoichiometric decomposition theory (Chen et al., 2014). Additionally, N+PK caused more severe

562 ecosystem dis-services, such as greenhouse gas emissions, than the 0.75N+PK treatment. Moreover,

563 compared with the N+PK treatment, the 0.75N+PK treatment increased the soil C:N ratio and

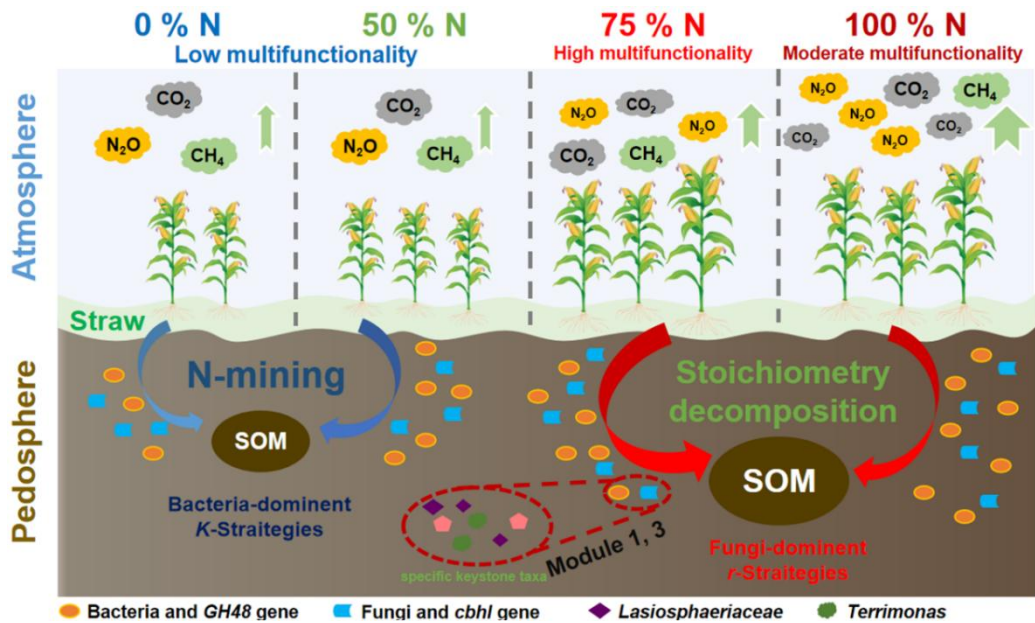
564 stimulated the microbial community functions of modules 1 and 3, *cbhI* gene abundance, and the

565 abundances of keystone taxa, which were significantly positively correlated with soil ecosystem

566 multifunctionality. The *Lasiosphaeriaceae*-driven module 1 and *Terrimonas*-driven module 3

567 communities may be involved in maintaining soil ecosystem multifunctionality. Our study suggests that

568 a 25% reduction in chemical N fertilizer after straw return is the optimal agronomic measure for

569 promoting ecosystem services in meadow soil on the Northeast China Plain.

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571 **Fig. 6** Graphical sketch of the changes in ecosystem services and potential microbial mechanisms in

572 response to different chemical N fertilizer application rates after straw return. N, nitrogen; SOM, soil

573 organic matter.

574 **5. Conclusion**

575   Straw return combined with different chemical N fertilizer application rates significantly changed

576 ecosystem services and dis-services. Collectively, our work indicates that compared with the N+PK

577 treatment, straw return with a 25% reduction in chemical N fertilizer has the potential to improve

578 ecosystem services by maintaining soil fertility, productivity, microbial biomass and function;

579 promoting straw decomposition and C and N release; and mitigating greenhouse gas emissions. The

580 0.75N+PK treatment achieved higher soil ecosystem multifunctionality than all the other treatments. In

581 addition, the N input level, straw biomass and soil C:N ratio can increase the abundance of the *cbhI* and

582 *GH48* genes, which may contribute to soil ecosystem multifunctionality.

583   Furthermore, changes in multiple soil ecosystem services were strongly associated with microbial

584 module communities and keystone taxa. The relationships between ecosystem services and microbial

585 traits confirmed that *Lasiosphaeriaceae*, which drives the function and structure of the module 1

586   community, promoted straw degradation and straw C and N release, while *Terrimonas*, which drives

587   the function and structure of the module 3 community, likely contributed to improved production under

588   the 0.75N+PK treatment. Therefore, a 25% reduction in chemical N fertilizer with straw return might

589   be a win–win strategy that not only produces considerable ecological benefits for the pedosphere and

590   atmosphere but also reduces fertilizer costs in meadow soil on the Northeast China Plain.

Author contributions

YD, LFW, and XHM designed the experiment; YD, HMC, ZN, WLZ, and YMW performed the measurements; YD, YMC, MXZ, and JYL analyzed the data; YD and MHC wrote the manuscript draft; YML, JYL and LFW reviewed and edited the manuscript.

Declaration of competing 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.

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Figure captions

Fig. 1 Fifteen cropland variables and multifunctionality index values under different N input levels after straw return. Abbreviations: N+PK, straw return plus regular inorganic N-P-K fertilizers; 0.75N+PK, straw return plus regular inorganic P-K with 25% N fertilizer reduction; 0.5N+PK, straw return plus regular inorganic P-K with 50% N fertilizer reduction; PK, straw return plus regular inorganic P-K without N fertilizer.

Fig. 2 Relationships of microbial module communities with soil ecosystem services and dis-services. Multitrophic network including multiple ecological modules. The colors of the nodes represent different ecological modules (a). OTU number proportions of bacteria and fungi (b). Proportions of edges linking bacteria to bacteria (B-B), bacteria to fungi (B-F) and fungi to fungi (F-F) in the major ecological modules (c). Links connecting specific module communities with soil ecosystem services and dis-services (d). * indicates significance at $P < 0.05$. Abbreviations: SOC, soil organic carbon; C:N, ratio of SOC content to total N content; N:P, ratio of total N content to total P content.

Fig. 3 Topological roles of microbial taxa and their effects on the soil multifunctionality index. The topological role of each OTU was determined according to the scatter plot of within-module connectivity (Z) and among-module connectivity (P) (a). Distribution of keystone taxa in each ecological module (b). Contributions of bacterial (c) and fungal OTUs (d) to the soil multifunctionality index. *, ** and *** indicate significance at $P < 0.05$, 0.01 and 0.001, respectively.

Fig. 4 Heatmap showing the close correlations of fertilizer (N input and straw return) and soil stoichiometry with microbial traits. *, ** and *** indicate significance at $P < 0.05$, 0.01 and 0.001,

respectively. Abbreviations: C:N, ratio of SOC content to total N content; N:P, ratio of total N content to total P content.

Fig. 5 Contribution of abiotic and biotic variables to the soil multifunctionality index. *, ** and *** indicate significance at P < 0.05, 0.01 and 0.001, respectively. Abbreviations: C:N, ratio of SOC content to total N content; N:P, ratio of total N content to total P content.

Fig. 6 Graphical sketch of the changes in ecosystem services and potential microbial mechanisms in response to different chemical N fertilizer application rates after straw return. N, nitrogen; SOM, soil organic matter.

Table 1 Abundances of genes encoding cellulose-degrading enzymes across different N fertilizer treatments after straw return

Treatment	*cbhI* gene abundance ($\times 10^6$ copies g^{-1} soil)	*GH48* gene abundance ($\times 10^7$ copies g^{-1} soil)	*cbhI*: *GH48* ratio
N+PK	4.75±0.16 a	1.68±0.01 a	0.28±0.01 a
0.75N+PK	4.95±0.19 a	1.60±0.04 a	0.31±0.02 a
0.5N+PK	4.01±0.12 b	1.54±0.08 a	0.26±0.03 b
PK	3.76±0.13 b	1.40±0.06 b	0.27±0.02 b

The results are presented as the means ± standard deviations (n = 3). Different lowercase letters after values indicate significant differences between each treatment, $P < 0.05$. N+PK, straw return plus regular inorganic N-P-K fertilizers; 0.75N+PK, straw return plus regular inorganic P-K with 25% N fertilizer reduction; 0.5N+PK, straw return plus regular inorganic P-K with 50% N fertilizer reduction; PK, straw return plus regular inorganic P-K without N fertilizer.