Soil Science Society of America Journal

Type of Contribution: Original research article

Date of Preparation:

Number of Text Pages: 28 pages

Number of Tables: 6 tables

Number of Figures: 4 figures

Title: The role of soil characteristics on temperature sensitivity of soil organic matter.

Authors: Michelle L. Haddix[1*], Alain F. Plante[2], Richard T. Conant[1], Johan Six[3], J. Megan Steinweg[1], Kim Magrini-Bair[4], Rhae A. Drijber[5], Sherri J. Morris[6], Eldor A. Paul[1]

Addresses: [1]Natural Resource Ecology Laboratory, Colorado State University, Fort Collins, CO 80523-1499; [2]Department of Earth & Environmental Science, University of Pennsylvania, Philadelphia, PA 19104-6316; [3]Department of Plant Sciences, University of California, Davis, CA 95616; [4]National Renewable Energy Laboratory, Golden, CO 80401; [5]Department of Agronomy and Horticulture, University of Nebraska, Lincoln, NE 68583-0915; [6]Biology Department, Bradley University, Peoria, IL 61625

*Corresponding author: Phone: (970) 491-6162; Fax: (970) 491-1965; E-mail: mlhaddix@nrel.colostate.edu

1   **The role of soil characteristics on temperature sensitivity of soil organic matter.**

2

3   **Abstract**

4   The uncertainty associated with how projected climate change will affect global carbon

5   (C) cycling could have a large impact on soil C stocks. The purpose of our study was to

6   determine how various soil decomposition and chemistry characteristics relate to SOM

7   temperature sensitivity. We accomplished this objective using long-term soil incubations

8   at three temperatures (15, 25, and 35°C) and pyrolysis molecular beam mass

9   spectrometry (py-MBMS) on twelve soils from six sites along a mean annual temperature

10   (MAT) gradient (2 to 25.6°C). We calculated Q_{10} values from the CO_2 respired in our

11   long-term incubation using the Q_{10-q} method and found the decomposition of the resistant

12   fraction to be more temperature sensitive with a Q_{10-q} of 1.95±0.08 for the labile fraction

13   and a Q_{10-q} of 3.33±0.04 for the resistant fraction. We used a two pool model (active and

14   slow) with first-order kinetics to fit our soil respiration data, along with a three pool

15   model for comparison purposes. We found that the two and three pool models

16   statistically fit the data equally well. The size of the active pool in these soils, calculated

17   using the two pool model, increased with incubation temperature and ranged from 0.1 to

18   12.8% of initial soil organic C. Sites with an intermediate MAT and lowest C:N ratio had

19   the largest active pool. Pyrolysis Molecular Beam Mass Spectrometry on these soils

20   indicated that there were chemical differences in the SOM between the land use

21   treatments which may have lead to differences found between the total amount of CO_2

22   respired and size of the active pool of the soils.

1. Introduction

Temperature effects on chemical reactions have been studied since the late 1800's, originating with Arrhenius and van't Hoff (Lloyd and Taylor, 1994); yet, there is still much debate on mechanistically how temperature regulates soil organic matter (SOM) decomposition. Soil organic matter contains two to three times as much C as the atmosphere (Davidson et al., 2000), with respiration of SOM by microbes contributing 50-75 Pg of CO_2-C to the atmosphere annually – approximately ten times the current annual emissions from burning fossil fuels (Schimel, 1995). Temperature is an important factor controlling SOM turnover and understanding how temperature affects SOM decomposition will allow us to better predict how global climate change will affect SOM stocks.

A Q_{10} of 1.5-2.0 or an Arrhenius type function, where the effective activation energy for respiration varies inversely with temperature, has commonly been used for the temperature response of SOM decomposition (Friedlingstein et al., 2006), and most terrestrial C models apply the same respiration-temperature relationship to each of the different SOM pools (Melillo et al., 1995; Burke, 2003; Friedlingstein et al., 2006). Contrary to common model formulations in which different types of SOM follow the same temperature decomposition relationship, many recent studies have found that

4

45   different SOM pools have different temperature responses, although consensus on their

46   apparent temperature sensitivities has not yet been reached (Davidson and Janssens,

47   2006). In addition, although determining the temperature sensitivity of SOM by

48   evaluating the Q_{10} response is commonly calculated using incubations of fixed durations,

49   the approach may generate incorrect values for temperature sensitivity due to

50   comparisons of SOM with different labilities at different temperatures (Leifeld and

51   Fuhrer, 2005; Reichstein et al., 2005; Conant et al., 2008), which further complicates

52   conclusions about SOM temperature sensitivity.

53   Understanding the temperature sensitivity of SOM decomposition is challenging

54   because soil organic matter is composed of many different organic C compounds, with

55   differing inherent kinetic properties (Davidson and Janssens, 2006). To simplify the

56   process of modeling SOM decomposition, this range of compounds is usually classified

57   into a small number of discrete, kinetically-defined pools with some portion of SOM

58   being labile and easily decomposable and the rest comprising one or more other pools

59   being less labile and decomposing slowly. In most decomposition models, temperature

60   effects are modeled as a decomposition rate multiplier for fixed SOM pools (Lloyd and

61   Taylor, 1994; Del Grosso et al., 2005). However, some recent studies have hypothesized

62   that temperature may actually alter the amount of substrate that would be considered

63   easily decomposable or labile (Zogg et al., 1997; Zak et al., 1999; Dalias et al., 2003;

64   Rasmussen et al., 2006). Such a response could be caused by temperature-driven shifts in

65   microbial community composition (Zogg et al., 1997; Zak et al., 1999) or in the selection

66   of compounds being decomposed (Andrews et al., 2000). A change in the microbially-

5

67 available SOM indicates that reaction rates may not be the only factor controlling

68 decomposition responses to altered temperatures.

69   The objective of this study was to reconcile the differences between the inherent

70 and apparent temperature sensitivity of SOM with differing labilities. To accomplish this

71 we determined the temperature sensitivity of a labile and more resistant soil fraction

72 using a long-term incubation on a set of soils along a mean annual temperature gradient

73 (MAT). At each point along our MAT we had two soils with different land uses as a way

74 to compare similar soils with differing amounts and types of SOM. We used respiration

75 response curves and pyrolysis molecular beam mass spectrometry to characterize the

76 SOM in each of our soils. By utilizing this multi-approach method we wanted to have a

77 better understanding of the apparent temperature sensitivity of SOM.

78

79 **2. Materials and Methods**

80 *2.1. Sample Sites*

81   Surface soil samples were collected from six sites along a mean annual

82 temperature gradient (2 to 25.6°C), each with a native and either cultivated (Indian Head,

83 SK; Mandan, ND; Akron, CO; and Waggoner Ranch, TX) or pasture (Alajuela, Costa

84 Rica and Nova Vida Ranch, Brazil) land use (Table 1). At each site, samples were

85 collected from three locations separated by several meters each (field replicate n = 3)

86 within each land use. Surface litter and above ground vegetation were cleared away prior

87 to sampling. Small pits were dug to a depth of 20 cm, and samples were collected from 0-

88 20 cm. Soils were packaged and transported to the laboratory, where rocks, surface litter

89 and root materials were removed while soil clods were broken by hand and passed

90 through a 2-mm sieve. Soil samples were then air-dried and stored at room temperature

91 until incubations began. Soil organic C (SOC) and total N concentrations were

92 determined with a LECO CHN-1000 autoanalyzer (LECO Corporation, St. Joseph, MI,

93 USA).

94

95 *2.2. Laboratory Incubation*

96 Four laboratory replicates from bulked field samples from each site and treatment

97 combination were incubated at 15, 25, and 35°C for 588 days. For each sample, 80 g of

98 soil were wetted up to 60% water filled pore space to optimize microbial activity (Linn

99 and Doran, 1984). Gravimetric soil moisture was periodically checked throughout the

100 incubation and water was added when water loss was greater than 5% of initial water

101 added. Samples were placed in sealed canning jars fitted with septa, along with

102 scintillation vials containing 20 ml of water to maintain humidity. Incubation starting

103 days were staggered by replicate and each replicate of a soil was measured on a different

104 day to take into account daily measurement variability. Soils were pre-incubated for

105 three days at 25°C and then four days at the respective incubation temperature prior to

106 measurements to allow the soil to equilibrate after wetting up (Paul et al., 2001).

107 Headspace gas samples were analyzed for CO_2 concentration using a Li-Cor LI-6252

108 IRGA (LI-COR Biosciences Lincoln, NE, USA). Jars were flushed with compressed

109 tank air regularly before CO_2 concentrations reached 5% to prevent CO_2 concentration

110 from inhibiting microbial activity. The CO_2 measurements were taken daily during the

111 first two weeks of the incubation, weekly for the next two weeks, and then every four

112 weeks thereafter, generating a total of 36 sampling times over the course of 588 days.

113

114 *2.3. Temperature Sensitivity*

115 Temperature sensitivity of SOM decomposition/mineralization was determined

116 using the Q_{10-q} method described by Conant et al. (2008a) utilizing the CO_2 respired from

117 our long-term incubations. The method involves determining the amount of time needed

118 for a given amount of C to be respired at a given temperature. The time required to

119 respire a given amount of C at two temperatures is then used to calculate a Q_{10} value.

120 There are two assumptions associated with this method (Conant et al., 2008a). The first

121 is that changes in decomposition rates during the incubation are due to changes in the

122 lability of the SOM being decomposed. The second is that the effect temperature has on

123 the sequence in which SOM compounds are decomposed is small relative to the effect of

124 temperature on decomposition rates. Soil microbial biomass has been found to decline

125 over time in long-term incubations (Follett et al., 2007), but this decline in biomass has

126 not been found to limit the microorganisms capacity to decompose organic matter in

127 long-term incubations (Follett et al., 2007; Steinweg et al., 2008). Because of this we

128 believe that our first assumption that changes in decomposition rates are due to changes

129 in SOM lability is reasonable. Q_{10-q} values were calculated for the initial 0.5% soil C

130 respired, which we considered the labile fraction, and the last 0.5% C respired, which we

131 considered the resistant fraction. Q_{10-q} values were calculated using the 25 and 35°C

132 incubations. The 15°C respiration data was not used due to the limited amount of C

8

133   respired by the Costa Rica pasture soil. Statistical comparisons of $Q_{10\text{-}q}$ values were done

134   by treating the six different sites as random variable replicates (PROC MIXED, SAS

135   Institute, Cary NC).

136

137   *2.4. Curve Fitting*

138   We used CO_2 respiration results from the 15, 25, and 35°C incubations for curve

139   fitting. The respiration results from the four laboratory replicates of each site-treatment-

140   temperature combination were averaged, and mean respiration rates were used to

141   determine pool size and decomposition rate constants by curve fitting. Pool size and

142   decomposition rate constants were determined using a two-pool model where the two

143   pools, active (C_a) and slow pool (C_s), sum to the total soil C (C_{soil}):

144    $$C_{soil} = C_a + C_s \tag{1}$$

145   We used a two-pool first-order equation (Andren and Paustian, 1987):

146    $$C_{cum}(t) = C_a(1 - e^{-k_a * t}) + C_s(1 - e^{-k_s * t}) \tag{2}$$

147   where C_{cum} (t) is the cumulative soil respiration at time t (μg C (g soil)$^{-1}$), C_a is the size of

148   the active fraction (μg C (g soil)$^{-1}$), and C_s is the size of the slow fraction (μg C (g soil)$^{-1}$).

149   The parameters k_a and k_s are the decomposition rate constants (day^{-1}) for the active and

150   slow pools, respectively. We utilized data from rate curves rather than cumulative

151   respiration because this minimized error accumulation through time (Hess and Schmidt,

152   1995). We used the following rate form of eqn. 2 from Paul et al. (2001) to determine

153   parameter estimates:

$$154 \qquad \frac{\Delta C_{cum}}{\Delta t} = C_a * k_a (e^{-k_a * t}) + C_s * k_s (e^{-k_s * t}) \qquad (3)$$

155   where $\Delta C_{cum}/\Delta t$ is in units of µg C (g soil)$^{-1}$ (day)$^{-1}$. The size of the active and slow pools

156   adds up to the total amount of C in the soil (C_{soil}) (eqn 1); causing C_s to be determined by

157   the difference of the total soil C and the active pool C. This method of curve fitting also

158   has the assumption that changes in respiration rate over the course of an incubation are

159   due to changes in SOM lability.

160   A three-pool model with first-order kinetics has been found to effectively describe

161   SOC dynamics (Paustian et al., 1992); because of this we also fit our respiration rate

162   curves to a three-pool first order equation. We used the same rate equation as the two-

163   pool model (eqn. 3) and included a resistant pool which was estimated to be fifty percent

164   of the total soil C. The decomposition rate for the resistant pool was estimated using a

165   field mean residence time of 500 years and a Q_{10} adjustment of 2 for the different

166   incubation temperatures. This calculation was only done for comparative purposes and

167   parameter estimates and subsequent temperature sensitivity calculations were done with

168   the two-pool equation.

169   Best fit parameters (C_a, k_a and k_s) for the two-pool model were estimated using

170   non-linear regression of the CO_2 evolved with time in SAS v9.2 PROC NLIN with the

171   Gauss method (SAS Institute, Cary NC). The only restriction imposed on parameters is

172   that the values had to be greater than zero. There were three instances (N. Dakota native

173   grassland 15℃, Colorado native grassland 15℃, and Colorado cultivated 15℃) where

174   the slow pool decomposition rate were not positive and these three samples were not used

175 in subsequent analysis. The errors associated with model parameters are standard error of

176 the model determined in SAS NLIN.

177

178 *2.5 Pyrolysis-molecular beam mass spectrometry (py-MBMS)*

179   Chemical composition of SOM was characterized using py-MBMS. Details of the

180 analytical method are provided in Magrini et al. (2002) and Hoover et al. (2002). Briefly,

181 two subsamples (~0.1 g) from each field replicate sample were weighed in quartz boats

182 and pyrolyzed in a reactor consisting of a quartz tube (2.5 cm inside diameter) with

183 helium flowing through at 5 l min^{-1} heated and maintained at 500°C. The molecular beam

184 system consisted of an ExtrelTM Model TQMS C50 mass spectrometer for both

185 pyrolysis and combustion vapor analysis. Mass spectral data from *m/z* 20 to 500 were

186 acquired on a Teknivent Vector 2TM data acquisition system using 22 eV electron

187 impact ionization and programmed storage in a personal computer. Repetitive scans

188 (typically one 480 amu scan s^{-1}) were recorded during the evolution of a pyrolysis wave

189 from each soil sample.

190   Overall, 36 samples were collected from the field (6 sites × 3 field replicates × 2

191 land uses). Two aliquots from each of these 36 were analyzed by py-MBMS, resulting in

192 72 total spectra. Two spectra from the analyses of samples from Saskatchewan and one

193 from North Dakota were, however, excluded from further analysis due to data quality

194 issues, leaving a total of 69 spectra.

195   Multivariate analysis has proven to be an important tool for pattern recognition in

196 pyrolysis mass spectrometry (Schulten et al., 1988). Signal intensities from individual

11

197 spectra (m/z 20-500) were normalized to 100% total ion intensity (TII, the sum of the

198 intensity for each m/z), and reduced data sets (m/z 57-500) were used in the multivariate

199 analyses to omit the small mass units typical of water, CO_2 and other volatiles. We used

200 principal component analysis (PCA) to group samples by similarity with samples having

201 similar chemical compositions being tightly grouped in a PCA score plot, while samples

202 with dissimilar and heterogeneous compositions were more scattered. Eight principal

203 components and full cross validation were used to build the PCA model to determine

204 whether SOM composition could be grouped by site or land use. Partial least squares

205 (PLS) regression analysis was used to predict the dependent variables (i.e., the model

206 estimates for respiration rates during incubation at 15, 25, and 35ºC) from our set of

207 independent variables (i.e., the signal intensities from the py-MBMS spectra). Two PLS

208 regressions were performed: one for the respiration rates early in the incubation when the

209 respiration is mostly from the active pool (day 5 of the incubation), and one for

210 respiration rates later in the incubation when the respiration is mostly from the slow pool

211 (day 225 of the incubation). Full cross-validation and Martens' uncertainty test were used

212 to determine statistically significant correlations between py-MBMS spectral data and the

213 biological properties of the samples. PLS analyses were performed iteratively to

214 determine which independent variables were significant based on Martens' uncertainty

215 test, and then subsequent PLS analyses were performed using only the significant

216 variables. This process was repeated until all independent variables were found to be

217 significant. All multivariate analyses were performed using the Unscrambler v.8.0

218 software package (CAMO Process AS, Oslo, Norway).

12

220 **3. Results**

221 *3.1. Site Comparisons*

222   Soil organic C (SOC) concentrations (0-20cm) ranged from 0.7 to 20.0% among

223 the six sites (Table 2). The Costa Rican soil is allophanic and consequently had much

224 higher SOC concentrations than the other five sites. SOC and total N concentrations

225 differed between land use within each site (except for Texas), with SOC and total N

226 concentrations decreasing after land-use conversion at Saskatchewan, N. Dakota,

227 Colorado, and Costa Rica.

228   Due to the large differences in SOC content among soils, the total amount of C

229 respired was normalized for the initial amount of SOC. After 588 days of incubation, the

230 cumulative amount of SOC respired was greater under warmer incubation temperatures

231 for all soils (Table 2). Overall there was a significant land use difference ($p<0.001$) for

232 the four sites where the native grassland was converted to wheat cultivation, with the

233 native soils on average respiring significantly more SOC than the cultivated soils, both in

234 absolute terms and after being normalized for total SOC content. Cumulative soil

235 respiration differences were not statistically significant for the native forest to pasture

236 land use conversion in the Costa Rica and Brazil sites ($p = 0.127$).

237

238 *3.3. Temperature Sensitivity*

239   In all but two instances the labile fraction had a smaller Q_{10-q} value than the

240 resistant fraction (Table 2) with N. Dakota cultivated and Brazil native forest being the

241   two exceptions. For the N. Dakota cultivated and the Brazil native forest the Q_{10-q} values

242   for the resistant fraction were much lower than the values for the resistant fraction of the

243   other soils. The Costa Rica pasture site had the highest Q_{10-q} values overall (table 2).

244   There did not appear to be a trend with MAT and Q_{10-q} values for the sites. When sites

245   were treated as replicates and Q_{10-q} values were pooled there was a significant difference

246   in Q_{10-q} values for the labile and resistant fractions (p = 0.0026) with the Q_{10-q} for the

247   labile fraction being 1.95±0.08 and the resistant fraction being 3.33±0.04. There was not

248   significant land use treatment effect (p = 0.127) or land use by SOM fraction effect (p =

249   0.338)

250

251   *3.2. Curve Fitting*

252   Respiration rates declined over time for all soils at all three incubation

253   temperatures with the two tropical sites, Costa Rica and Brazil, having a much more rapid

254   decline in respiration rates early in the incubation compared to the other four sites (Fig.

255   1). Respiration rates leveled off for all soils by the end of the incubation, but differences

256   in the respiration rates among the three incubation temperatures were still apparent at the

257   end of the incubation (Fig. 1).

258   Overall the two-pool equation fit the respiration data fairly well; an exception to

259   this was the Costa Rica native forest soil at 35ºC where the two-pool equation

260   overestimated the respiration rate later in the incubation (Fig. 1). We also fit the data to a

261   three-pool first order equation, described by Paul et al. (2001), where the third pool was

262   50% of the total SOC and the turnover for the third pool was estimated to be 500 years.

263   We found that both the two and three-pool equations statistically fit the data equally well.

264   Although estimates of the active pool size and decomposition rate were not statistically

265   different between the two-pool and three-pool equation, the three-pool equation

266   consistently had a smaller active pool compared to the two-pool equation by an average

267   of 5% and the three-pool equation consistently had a higher decomposition rate of the

268   active pool compared to the two-pool equation by an average of 6% (Table 3). We used

269   the simpler two-pool model on all subsequent analysis, realizing that the resistant pool

270   contributes only slightly to the respiration of the slow pool because of its high mean

271   residence time.

272   The native grassland Colorado soil had the largest active pool, comprising

273   between 9.4 and 14.0% of total SOC, while the Costa Rica native forest soil had the

274   smallest active pool, comprising between 0.1% and 1.0% of total SOC (Table 4). In the

275   native grassland/cultivated sites (Saskatchewan, N. Dakota, Colorado, and Texas) the

276   native treatment had a significantly larger active pool than the cultivated treatment ($p =$

277   0.003). The size of the active pool at 25°C appeared to be negatively correlated with the

278   C:N ratio of the soil ($r^2 = 0.34$, $p = 0.045$ for all soils) (Fig. 2). The relationship between

279   the active fraction and C:N was improved when the Colorado cultivated and Brazil native

280   forest soils were removed (r^2=0.84, p=0.0002), which appeared to behave differently than

281   the other soils.

282   Since there were no restrictions placed on the size of the pools, pool sizes were

283   allowed to vary with temperature if it provided the best fit of the data. In all but four

284   instances (N. Dakota cultivated 35°C, Texas cultivated 25°C, Brazil native forest 25°C,

285 Costa Rica pasture 25°C) there was an increase in active pool size with temperature. For

286 the four soils where the active pool size declined with increasing temperature, it was

287 likely due to high initial respiration rates that quickly declined over time. The

288 decomposition rates of the slow pool increased with increasing incubation temperature

289 for all of the soils and the decomposition rate for the active pool increased with

290 increasing temperature in only half the instances (Table 4).

291

292 *3.4. Soil organic matter composition*

293   A semi-empirical quantification of SOM composition can be achieved by

294 assigning the relative intensity of individual mass signals from the py-MBMS to a set of

295 previously identified marker signals associated with several classes of compounds (Sorge

296 et al., 1993; Schulten, 1996). A large proportion of the mass spectra of each sample could

297 be classified, leaving only $6.7 \pm 2.5\%$ of TII unidentified (Table 5). The volatile fraction

298 (m/z 20-56) represented $50.9 \pm 4.5\%$ of the total signal intensity, dominated by m/z 44.

299 The largest classes of identified compounds were various N-containing compounds (11.9

300 $\pm 1.8\%$ of TII) and carbohydrates ($10.6 \pm 2.6\%$ of TII). The various N-containing

301 compounds are identified by Schulten (1996) as heterocyclic N-containing compounds,

302 but the heterocyclic N is thought to be formed by the pyrolysis process (Sharma et al.,

303 2003). Peptides, phenols and lignin monomers, alkyl aromatics, and lipids were also

304 significant contributors (in decreasing order), while sterols and lignin dimers each

305 contributed < 1% of TII (Table 5). The conversion of native grasslands to agriculture

306   causes a decline in carbohydrates, but the change in carbon compounds with conversion

307   from native forest to pasture was not consistent for the other two sites (Table 5).

308   In many of the sites there were differences in the abundance of chemical

309   compounds with py-MBMS between the native and cultivated/pasture treatments. Figure

310   3 illustrates the differences in chemical composition between the native and cultivated

311   soils for the Saskatchewan site, as an example. The Saskatchewan native grassland had a

312   greater abundance of the lower m/z compounds especially m/z 57 and 96 which are

313   associated with carbohydrates and m/z 67 and 81 which are associated with various

314   nitrogen compounds. The cultivated soil had a greater abundance of higher molecular

315   weight compounds, many of which are associated with lipids, lignin, and alkyl aromatics.

316   Principal components analysis was unable to significantly distinguish SOM

317   composition when all samples were analyzed together (Fig. 4). The first four principal

318   components combined explained only 43% of the variance. Taken together, PCA scores

319   of samples were not tightly grouped on the basis of site or land use, with the exception

320   that native forest and grassland samples appear to be more tightly grouped than cultivated

321   samples. While there was minor separation of samples by site along the PC 1 axis, SOM

322   composition from those sites was comparatively heterogeneous. However, significant

323   clustering was observed on the basis of land-use treatment when sites were analyzed

324   individually (data not shown), although there were no consistent trends across sites. The

325   PCA score results suggest that SOM composition in the Costa Rica samples was highly

326   homogeneous. PCA loadings were dominated by low molecular weight and odd-

17

327   numbered m/z fragments, which are likely derived from carbohydrate, amino acid and

328   peptide side-chains (data not shown).

329   Sequential application of the Martens' uncertainty test found a small number of

330   m/z values that were significantly correlated to respiration rates at day 5 of the incubation

331   (Table 6). PLS regression using this reduced set of independent variables was able to

332   explain 65% of the variance using the first four components. Respiration rates at day-5 of

333   the incubation were highly correlated with low molecular components associated

334   primarily with carbohydrates, peptides and various N compounds. Regression against

335   respiration rates at day 225 of the incubation resulted in more m/z values that were

336   significantly correlated (Table 6), but each correlation coefficient was smaller than those

337   for day 5. PLS regression of the reduced set of independent variables was able to explain

338   59% of the variance using the first four components. The additional m/z values were

339   generally higher molecular weights. Some were associated with phenols and lignin

340   monomers, lignin dimmers, and lipids, but many were not identified by Schulten (1996),

341   Hempfling & Schulten (1990), or Gillespie et al. (2009) as marker signals for specific

342   compounds classes.

343

344   **4. Discussion**

345   During our incubation the total amount of SOC respired increased with warmer

346   incubation temperatures for all twelve soils. Similar responses across the MAT range of

347   2 to 25.6°C (Table 2) could suggest that increasing global temperatures could affect soils

348   equally. The low respiration rates per unit SOC for the Costa Rica soils were likely due

349   to the unique mineral composition. Protective effects of amorphous aluminum, iron, and

350   allophanic material have been shown to contribute to the much higher SOM

351   concentrations found in these types of soils (Munevar and Wollum, 1977; Boudot et al.,

352   1986; Martin and Haider, 1986; McKeague, 1986). The characteristics that cause SOM

353   accumulation also likely cause their minimal loss of SOC upon incubation (Boudot et al.,

354   1988). However, the relative temperature response of the Costa Rica soil did not differ

355   from the other soils.   Respiration rates declined over time in all the soils with the

356   differences in respiration rates between temperatures still apparent at the end of the

357   incubation. Correlations from PLS regression analysis of the py-MBMS products versus

358   the respiration rates could indicate that higher molecular weight compounds, and

359   therefore more resistant SOM, were being utilized in the later stages of the incubation.

360   These correlations support the first assumption of the Q_{10-q} method, that changes in

361   decomposition rates are driven in changes in SOM lability.

362   We found in most instances the resistant SOM was more temperature sensitive

363   than the labile SOM. We utilized the same method of determining temperature

364   sensitivity of SOM decomposition as described by Conant et al. (2008a) along with two

365   of the same sites (Colorado and Texas) and got similar results with an expanded set of

366   sites. The results found by us and Conant et al. (2008a) are consistent with multiple other

367   studies (Bosatta and Agren, 1999; Bol et al., 2003; Fierer et al., 2005; Knorr et al., 2005).

368   However, it is important to point out that there were two soils in our study that did not

369   follow this overall trend and in those two soils the resistant fraction had a lower Q_{10-q}

370   value than the labile fraction and the Q_{10-q} value of the resistant fraction was also much

371   lower than the $Q_{10\text{-}q}$ value determined for the resistant fraction of the other soils. Our

372   results illustrate how a study that utilizes a single soil for determining temperature

373   sensitivity of SOM decomposition could possibly come to a different conclusion with

374   regards to SOM temperature sensitivity.

375   The estimated size of the active pool for all the sites ranged from 0.1 to 14.0% of

376   initial SOC (Table 4). This range for the active pool is slightly wider than results found

377   by Rey and Jarvis (2006), which ranged from 0.27% to 11.4%. Many other studies have

378   estimates that fall with in these ranges (Collins et al., 2000; Haile-Mariam et al., 2000;

379   Cochran et al., 2007). Our wider range of estimates for the active pool is likely due to the

380   wide range of soils and incubation temperatures utilized. The two central sites, Texas

381   and Colorado, respired the most total SOC by the end of the incubation and had the

382   largest active pools, contrary to the comparison of forested tropical soils and dry forested

383   temperate soils by Trumbore (1993) in which the tropical soils were comprised of more

384   labile C in the upper 22 cm than temperate soils. The larger active pools in our temperate

385   soils and higher cumulative respiration could be due to the greater amount of N in the

386   soils, which indicates a higher proportion of proteinaceous constituents. Our results are

387   consistent with Thomsen et al. (2008), who found the C:N ratio to be an indicator of the

388   decomposability of the SOM in a soil, with soils having lower C:N ratios having greater

389   CO_2 evolution up to a certain threshold.

390   Temperature-induced changes in pool sizes could be an indication that warmer

391   temperatures may enable microbes to quickly decompose a larger portion of SOM.

392   Increased labile pool size with temperature evidenced in this study indicates that at

20

393 warmer temperatures SOM otherwise unavailable to microbes at lower temperatures

394 becomes available for decomposition. This could be attributed to shifts in microbial

395 community composition at different temperatures (Zogg et al., 1997; Zak et al., 1999),

396 changes is substrate use (Andrews et al., 2000; Schimel and Mikan, 2005), or the

397 overcoming of biochemical resistance of SOM by microbes (Conant et al., 2008b).

398   We had utilized different land use treatments at our sites as a way to compare

399 soils with the same MAT but varying amounts and types of SOM. For the four native

400 grassland/cultivated sites the native grassland treatments respired a greater amount of

401 total soil C and had larger active pools then their cultivated counterparts. The native

402 grassland soils had greater percentages of carbohydrates as found by py-MBMS, which

403 could be the reason for the greater respiration and larger active pool. Plante et al. (2009)

404 found that cultivation resulted in significant decreases in carbohydrates, peptides, and

405 phenols, also utilizing py-MBMS. Surprisingly, the decline in carbohydrates with

406 cultivation did not result in significant trends in Q_{10-q} values with land use. Our MAT

407 gradient did not produce consistent trends with regards to temperature sensitivity, also. It

408 may have been that differences in soil characteristics among sites were too great to

409 elucidate trends with MAT.

410   The size of our estimated active pool did increase with warmer incubation

411 temperatures for most soils indicating that there may be changes in microbial community

412 or function with temperature that should be considered in model incorporation. Our

413 results indicate that although individual soils may have varying apparent temperature

414 sensitivities for labile versus more resistant SOM depending on the inherent

415 characteristics of the soil, overall the more resistant SOM tends to be more temperature

416 sensitive. We were unable to link differences between inherent and apparent temperature

417 sensitivity of SOM to MAT, land use, or the chemical composition of SOM. This is an

418 area that requires more investigation and is necessary to better model temperature effects

419 on SOM decomposition.

420

421 **References**

422 Andrén, O., Paustian, K., 1987. Barley straw decomposition in the field - a comparison of

423   models. Ecology 68,1190-1200.

424 Andrews, J.A., Matamala, R., Westover, K.M., Schlesinger, W.H., 2000. Temperature

425   effects on the diversity of soil heterotrophs and the delta C-13 of soil-respired

426   CO_2. Soil Biology & Biochemistry 32, 699-706.

427 Black, A.L., and D. L. Tanaka, 1997. A conservation tillage-cropping systems study in

428   the Northern Great Plains of the United States. In: Paul, E.A. (Ed.). Soil organic

429   matter in temperate agroecosystems. CRC Press, Inc.335-342.

430 Bol, R., Bolger, T., Cully, R., Little, D., 2003. Recalcitrant soil organic materials

431   mineralize more efficiently at higher temperatures. Journal of Plant Nutrition and

432   Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde 166, 300-307.

433 Bosatta, E., Agren, G.I., 1999. Soil organic matter quality interpreted thermodynamically.

434   Soil Biology & Biochemistry 31, 1889-1891.

435 Boudot, J.P., Belhadj, B.A., Chone, T., 1986. Carbon Mineralization in Andosols and

436   Aluminum-Rich Highland Soils. Soil Biology & Biochemistry 18, 457-461.

437   Boudot, J.P., Brahim, A.B.H., Chone, T., 1988. Dependence of Carbon and Nitrogen

438   Mineralization Rates Upon Amorphous Metallic Constituents and Allophanes in

439   Highland Soils. Geoderma 42, 245-260.

440   Burke, I., J. P. Kaye, S. P. Bird, S. A. Hall, R. L. McCulley, and G. L. Sommerville,

441   2003. Evaluating and testing models of terrestrial biogeochemistry: The role of

442   temperature in controlling decomposition. In: Canham, C., J. Cole, W. Lauenroth

443   (Ed.). Models in Ecosystem Science. Princeton University Press, Princeton, N. J.,

444   pp. 225-253.

445   Campbell, C.A., G. P. Lafond, A. P. Moulin, L. Townley-Smith, and R. P. Zentner, 1997.

446   Crop production and soil organic matter in long-term crop rotations in the sub-

447   humid Northern Great Plains of Canada. In: Paul, E.A. (Ed.). Soil organic matter

448   in temperate agroecosystems. CRC Press, Inc.297-315.

449   Cerri, C.E.P., Paustian, K., Bernoux, M., Victoria, R.L., Melillo, J.M., Cerri, C.C., 2004.

450   Modeling changes in soil organic matter in Amazon forest to pasture conversion

451   with the Century model. Global Change Biology 10, 815-832.

452   Cochran, R.L., Collins, H.P., Kennedy, A., Bezdicek, D.F., 2007. Soil carbon pools and

453   fluxes after land conversion in a semiarid shrub-steppe ecosystem. Biology and

454   Fertility of Soils 43, 479-489.

455   Collins, H.P., Elliott, E.T., Paustian, K., Bundy, L.C., Dick, W.A., Huggins, D.R.,

456   Smucker, A.J.M., Paul, E.A., 2000. Soil carbon pools and fluxes in long-term

457   corn belt agroecosystems. Soil Biology & Biochemistry 32, 157-168.

458   Conant, R.T., Drijber, R.A., Haddix, M.L., Parton, W.J., Paul, E.A., Plante, A.F., Six, J.,

459   Steinweg, J.M., 2008a. Sensitivity of organic matter decomposition to warming

460   varies with its quality. Global Change Biology 14, 868-877.

461   Conant, R.T., Steinweg, J.M., Haddix, M.L., Paul, E.A., Plante, A.F., Six, J., 2008b.

462   Experimental warming shows that decomposition temperature sensitivity

463   increases with soil organic matter recalcitrance. Ecology 89, 2384-2391.

464   Dalias, P., Kokkoris, G.D., Troumbis, A.Y., 2003. Functional shift hypothesis and the

465   relationship between temperature and soil carbon accumulation. Biology and

466   Fertility of Soils 37, 90-95.

467   Davidson, E.A., Janssens, I.A., 2006. Temperature sensitivity of soil carbon

468   decomposition and feedbacks to climate change. Nature 440, 165-173.

469   Davidson, E.A., Trumbore, S.E., Amundson, R., 2000. Biogeochemistry - Soil warming

470   and organic carbon content. Nature 408, 789-790.

471   Del Grosso, S.J., Parton, W.J., Mosier, A.R., Holland, E.A., Pendall, E., Schimel, D.S.,

472   Ojima, D.S., 2005. Modeling soil CO2 emissions from ecosystems.

473   Biogeochemistry 73, 71-91.

474   Fierer, N., Craine, J.M., McLauchlan, K., Schimel, J.P., 2005. Litter quality and the

475   temperature sensitivity of decomposition. Ecology 86, 320-326.

476   Fissore, C., Giardina, C.P., Swanston, C.W., King, G.M., Kolka, R.K., 2009. Variable

477   temperature sensitivity of soil organic carbon in North American forests. Global

478   Change Biology 15, 2295-2310.

479   Follett, R.F., Paul, E.A., Pruessner, E.G., 2007. Soil carbon dynamics during a long-term

480   incubation study involving C-13 and C-14 measurements. Soil Science 172, 189-

481   208.

482   Friedlingstein, P., Cox, P., Betts, R., Bopp, L., Von Bloh, W., Brovkin, V., Cadule, P.,

483   Doney, S., Eby, M., Fung, I., Bala, G., John, J., Jones, C., Joos, F., Kato, T.,

484   Kawamiya, M., Knorr, W., Lindsay, K., Matthews, H.D., Raddatz, T., Rayner, P.,

485   Reick, C., Roeckner, E., Schnitzler, K.G., Schnur, R., Strassmann, K., Weaver,

486   A.J., Yoshikawa, C., Zeng, N., 2006. Climate-carbon cycle feedback analysis:

487   Results from the (CMIP)-M-4 model intercomparison. Journal of Climate 19,

488   3337-3353.

489   Gillespie, A.W., Walley, F.L., Farrell, R.E., Leinweber, P., Schlichting, A., Eckhardt,

490   K.U., Regier, T.Z., Blyth, R.I.R., 2009. Profiling Rhizosphere Chemistry:

491   Evidence from Carbon and Nitrogen K-Edge XANES and Pyrolysis-FIMS. Soil

492   Science Society of America Journal 73, 2002-2012.

493   Haile-Mariam, S., Cheng, W., Johnson, D.W., Ball, J.T., Paul, E.A., 2000. Use of carbon-

494   13 and carbon-14 to measure the effects of carbon dioxide and nitrogen

495   fertilization on carbon dynamics in ponderosa pine. Soil Science Society of

496   America Journal 64, 1984-1993.

497   Halvorson, A. D., Vigil M.F., Peterson G.A., Elliot E.T., 1997. Long-Term Tillage and

498   Crop Residue Management Study at Akron, Colorado. In: E. A. Paul (Eds.), Soil

499   organic matter in temperate agroecosystems. CRC Press, Inc, pp. 361-370.

500 Hempfling, R., Schulten, H.R., 1990. Chemical Characterization of the Organic-Matter in

501   Forest Soils by Curie-Point Pyrolysis GC MS and Pyrolysis Field-Ionization

502   Mass-Spectrometry. Organic Geochemistry 15, 131-145.

503 Hess, T.F., Schmidt, S.K., 1995. Improved Procedure for Obtaining Statistically Valid

504   Parameter Estimates from Soil Respiration Data. Soil Biology & Biochemistry 27,

505   1-7.

506 Hoover, C.M., Magrini, K.A., Evans, R.J., 2002. Soil carbon content and character in an

507   old-growth forest in northwestern Pennsylvania: a case study introducing

508   pyrolysis molecular beam mass spectrometry (py-MBMS). Environmental

509   Pollution 116, S269-S275.

510 Knorr, W., Prentice, I.C., House, J.I., Holland, E.A., 2005. Long-term sensitivity of soil

511   carbon turnover to warming. Nature 433, 298-301.

512 Leifeld, J., Fuhrer, J., 2005. The temperature response of CO_2 production from bulk soils

513   and soil fractions is related to soil organic matter quality. Biogeochemistry 75,

514   433-453.

515 Linn, D.M., Doran, J.W., 1984. Effect of Water-Filled Pore-Space on Carbon-Dioxide

516   and Nitrous-Oxide Production in Tilled and Nontilled Soils. Soil Science Society

517   of America Journal 48, 1267-1272.

518 Lloyd, J., Taylor, J.A., 1994. On the Temperature-Dependence of Soil Respiration.

519   Functional Ecology 8, 315-323.

520 Magrini, K.A., Evans, R.J., Hoover, C.M., Elam, C.C., Davis, M.F., 2002. Use of

521   pyrolysis molecular beam mass spectrometry (py-MBMS) to characterize forest

522   soil carbon: method and preliminary results. Environmental Pollution 116, S255-

523   S268.

524   Martin, J.P., Haider, K., 1986. Influence of mineral colloids on turn-over rates of soil

525   organic carbon. In: Huang, P.M. (Ed.). Interactions of soil minerals with natural

526   organics and microbes. The Soil Science Society of America, Madison, Wis.,

527   USA, pp. 283-304.

528   Martin, R.E., Asner, G.P., Ansley, R.J., Mosier, A.R., 2003. Effects of woody vegetation

529   encroachment on soil nitrogen oxide emissions in a temperate savanna. Ecological

530   Applications 13, 897-910.

531   McKeague, J.A., M. V. Cheshire, F. Andreux, and J. Berthelin, 1986. Organo-mineral

532   complexes in relation to pedogenesis. In: Huang, P.M. (Ed.). Interactions of soil

533   minerals with natural organics and microbes. The Soil Science Society of

534   America, Madison, Wis., USA, pp. 549-592.

535   Melillo, J.M., Borchers, J., Chaney, J., Fisher, H., Fox, S., Haxeltine, A., Janetos, A.,

536   Kicklighter, D.W., Kittel, T.G.F., McGuire, A.D., McKeown, R., Neilson, R.,

537   Nemani, R., Ojima, D.S., Painter, T., Pan, Y., Parton, W.J., Pierce, L., Pitelka, L.,

538   Prentice, C., Rizzo, B., Rosenbloom, N.A., Running, S., Schimel, D.S., Sitch, S.,

539   Smith, T., Woodward, I., 1995. Vegetation Ecosystem Modeling and Analysis

540   Project - Comparing Biogeography and Biogeochemistry Models in a

541   Continental-Scale Study of Terrestrial Ecosystem Responses to Climate-Change

542   and Co2 Doubling. Global Biogeochemical Cycles 9, 407-437.

543 Munevar, F., Wollum, A.G., 1977. Effects of Addition of Phosphorus and Inorganic

544   Nitrogen on Carbon and Nitrogen Mineralization in Andepts from Colombia. Soil

545   Science Society of America Journal 41, 540-545.

546 Paul, E.A., S. J. Morris, S. Böhm, 2001. The Determination of Soil C Pool Sizes and

547   Turnover Rates: Biophysical Fractionation and Tracers. In: Lal, R., J. M. Kimble,

548   R. F. Follett, B. A. Stewart (Ed.). Assessment Methods for Sol Carbon. Lewis

549   Publishers, Boca Raton, pp. 193-206.

550 Paustian, K., Parton, W.J., Persson, J., 1992. Modeling Soil Organic-Matter In Organic-

551   Amended and Nitrogen-Fertilized Long-Term Plots. Soil Science Society of

552   America Journal 56, 476-488.

553 Plante, A.F., Magrini-Bair, K., Vigil, M., Paul, E.A., 2009. Pyrolysis-molecular beam

554   mass spectrometry to characterize soil organic matter composition in chemically

555   isolated fractions from differing land uses. Biogeochemistry 92, 145-161.

556 Rasmussen, C., Southard, R.J., Horwath, W.R., 2006. Mineral control of organic carbon

557   mineralization in a range of temperate conifer forest soils. Global Change Biology

558   12, 834-847.

559 Reichstein, M., Katterer, T., Andrén, O., Ciais, P., Schulze, E.D., Cramer, W., Papale, D.,

560   Valentini, R., 2005. Temperature sensitivity of decomposition in relation to soil

561   organic matter pools: critique and outlook. Biogeosciences 2, 317-321.

562 Rey, A., Jarvis, P., 2006. Modeling the effect of temperature on carbon mineralization

563   rates across a network of European forest sites (FORCAST). Global Change

564   Biology 12, 1894-1908.

565   Schimel, J.P., Mikan, C., 2005. Changing microbial substrate use in Arctic tundra soils

566   through a freeze-thaw cycle. Soil Biology & Biochemistry 37, 1411-1418.

567   Schimel, J.P., 1995. CO_2 and the carbon cycle. In: Houghton, J.T. (Ed.). Climate change,

568   1994: radiative forcing of climate change and an evaluation of the IPCC IS92

569   emission scenarios. Cambridge University Press, Cambridge [England]; New

570   York, pp. 35-71.

571   Schulten, H.R., 1996. Direct pyrolysis-mass spectrometry of soils: a novel too in

572   agriculture, ecology, forestry, and soil science. In: Boutton, T.W., Yamasaki, S.-i.

573   (Eds.). Mass spectrometry of soils. M. Dekker, New York, pp. 373-436.

574   Schulten, H.R., Hempfling, R., Zech, W., 1988. Discriminating horizons in a modern

575   profile by field-ionization mass-spectrometery and pattern-recognition. Geoderma

576   41, 211-222.

577   Sharma, R.K., Chan, W.G., Seeman, J.I.Hajaligol, M.R., 2003. Formation of low

578   molecular weight heterocycles and polycyclic aromatic compounds (pacs) in the

579   pyrolysis of alpha-amino acids. Journal of Analytical and Applied Pyrolysis

580   66,97-121.

581   Sorge, C., Muller, R., Leinweber, P., Schulten, H.R., 1993. Pyrolysis mass-spectrometry

582   of whole soils, soil particle-size fractions, litter materials and humic substances -

583   statistical evaluation of sample weight, residue, volatilized matter and total ion

584   intensity. Fresenius Journal of Analytical Chemistry 346, 697-703.

585   Thomsen, I.K., Petersen, B.M., Bruun, S., Jensen, L.S., Christensen, B.T., 2008.

586   Estimating soil C loss potentials from the C to N ratio. Soil Biology &

587   Biochemistry 40, 849-852.

588   Trumbore, S.E., 1993. Comparison of Carbon Dynamics in Tropical and Temperate Soils

589   Using Radiocarbon Measurements. Global Biogeochemical Cycles 7, 275-290.

590   Zak, D.R., Holmes, W.E., MacDonald, N.W., Pregitzer, K.S., 1999. Soil temperature,

591   matric potential, and the kinetics of microbial respiration and nitrogen

592   mineralization. Soil Science Society of America Journal 63, 575-584.

593   Zogg, G.P., Zak, D.R., Ringelberg, D.B., MacDonald, N.W., Pregitzer, K.S., White,

594   D.C., 1997. Compositional and functional shifts in microbial communities due to

595   soil warming. Soil Science Society of America Journal 61, 475-481.

596   Table 1. Characteristics of the six locations, sampled in the spring of 2005, used in our long-term incubation

Site	GPS location	MAT/ MAP	Soil Taxonomy	Treatment	% Clay	Vegetation	Year converted to present land use	Citation
Indian Head, Saskatchewan ARGCN	50.533 N -103.517 W	2°C 421 mm	Udic Boroll	Native grassland	50	Grassland containing predominantly cool season grasses	1957	Campbell et al. 1997
				Cultivated	61	Spring-wheat-based rotations		
Mandan, North Dakota NGPRL	46.767 N -100.917 W	5°C 402mm	Typic Argiboroll	Native grassland	28	Warm mixed grass prairie	1984	Black & Tanaka 1997
				Cultivated	33	Continuous spring wheat		
Akron, Colorado CGPRS	40.150 N -103.150 W	9.2°C 420 mm	Aridic Paleustoll	Native grassland	23	Grassland with predominantly C_4 grasses	1957	Halvorson et al. 1997
				Cultivated	28	Continuous wheat		
Vernon, Texas Waggoner Ranch	33.939 N -99.413 W	17°C 665 mm	Typic Paleustoll	Native grassland	31	Grassland with a mix of C_4 and C_3 grasses	N/A	Martin et al. 2003
				Cultivated	44	Continuous wheat with conventional tillage		
Alajuela, Costa Rica Alajuela Research Station	N/A	20°C N/A	Hydric Melanudand	Native forest	9	Tropical forest with predominantly C_3 species	1979	N/A
				Pasture	12	C_4 warm season grasses		
Rondônia, Brazil Nova Vida Ranch	10.168 S -62.824 W	25.6°C 2200mm	Paleudult & Kandiuldult	Native forest	30	Open humid tropical forest with predominantly C_3 species	1972	Cerri et al. 2004
				Pasture	25	C_4 warm season grasses		

597   MAT, mean annual temperature; MAP, mean annual precipitation.

31

598 Table 2. Soil C and N content, C:N ratio, cumulative respiration after 588 days of incubation at 15ºC, 25ºC, and 35ºC, and Q_{10-q}
599 calculated for the 35/25°C comparison using the cumulative respiration after 588 days for the six sites and land-use types examined in
600 the cross site comparison. (mean ± 1 standard deviation, n=4)

Site[†]	Land Use[‡]	SOC (%)	Total N (%)	C:N	Cumulative Respiration (% total soil C)			Q_{10-q}	
					15 ℃	25 ℃	35 ℃	labile [§]	resistant[¶]
SK	NG	3.71 ± 0.09	0.358 ± 0.008	10.4	6.52 ± 0.37	10.40 ± 0.60	14.37 ± 0.23	1.6 ± 0.0	2.5 ± 0.2
SK	C	2.29 ± 0.09	0.204 ± 0.021	11.2	4.33 ± 0.14	7.73 ± 0.53	13.05 ± 0.50	2.0 ± 0.1	3.3 ± 1.3
ND	NG	3.24 ± 0.06	0.293 ± 0.004	11.0	3.93 ± 0.30	8.14 ± 0.68	15.03 ± 2.90	1.7 ± 0.1	2.7 ± 0.2
ND	C	2.80 ± 0.24	0.240 ± 0.004	11.7	5.17 ± 0.16	9.17 ± 0.44	11.32 ± 1.06	1.5 ± 0.1	1.3 ± 0.2
CO	NG	1.16 ± 0.08	0.135 ± 0.003	8.6	9.64 ± 0.65	17.93 ± 0.53	30.20 ± 2.39	1.5 ± 0.1	2.8 ± 0.3
CO	C	0.69 ± 0.02	0.100 ± 0.002	6.9	7.45 ± 0.25	13.09 ± 1.58	24.04 ± 2.58	2.2 ± 0.2	4.6 ± 1.4
TX	NG	1.12 ± 0.02	0.129 ± 0.006	8.7	9.60 ± 0.46	14.96 ± 1.10	24.36 ± 0.43	2.0 ± 0.2	3.4 ± 0.5
TX	C	1.02 ± 0.04	0.125 ± 0.004	8.1	8.62 ± 0.20	15.61 ± 0.68	23.22 ± 1.60	1.6 ± 0.1	3.1 ± 0.2
CR	NF	20.02 ± 0.62	1.660 ± 0.045	12.1	2.04 ± 0.10	4.21 ± 0.27	9.70 ± 0.53	1.9 ± 0.2	4.1 ± 0.4
CR	P	14.16 ± 0.19	1.045 ± 0.019	13.5	1.29 ± 0.05	2.68 ± 0.07	5.65 ± 0.17	2.9 ± 0.3	6.5 ± 0.4
BR	NF	1.06 ± 0.02	0.109 ± 0.006	9.7	5.72 ± 0.54	11.81 ± 0.74	19.50 ± 2.28	2.2 ± 0.3	1.4 ± 0.3
BR	P	1.41 ± 0.02	0.133 ± 0.011	10.6	6.76 ± 0.36	10.65 ± 0.45	21.60 ± 0.97	2.4 ± 0.4	4.2 ± 0.6

601 [†] SK, Saskatchewan; ND, North Dakota; CO, Colorado; TX, Texas; CR, Costa Rica; BR, Brazil

602 [‡] NG, native grassland; NF, native forest; C, cultivated; P, pasture

603 [§] Labile was considered the first 0.5% SOC respired in the incubation

604 [¶] Resistant was considered the last 0.5% SOC respired in the incubation

605 Table 3. Parameter estimates averaged across all soils and temperatures and fit estimates for the two-pool and three-pool models for

606 the six sites and land use types examined in the cross site comparison.

Model	Active Pool (μg C g soil^{-1})	Active Pool Decomp. Rate (day^{-1})	Slow Pool (μg C g soil^{-1})	Slow Pool Decomp. Rate (day^{-1})	Resistant Pool (μg C g soil^{-1})	Resistant Pool Decomp. Rate (day^{-1})	R_a^2
2 pool	1060	2.85E-02	42840	1.36E-04	.	.	0.83
3 pool	1002	3.03E-02	20948	3.10E-04	21950	1.76E-05	0.83

607 R_a^2, adjusted r-square

608

609

33

Table 4. Size of the active pool and active and slow pool decomposition rates at 15℃, 25℃, and 35℃ for the two-pool model for the

six sites and land-use types examined in the cross site comparison. Error is model standard error.

Site [†]	Land use [‡]	Inc. Temp (℃)	Active Pool (% total soil C)	Active Pool Decomp. Rate (day^{-1})	Slow Pool Decomp. Rate (day^{-1})
SK	NG	15	3.7 ± 1.3	6.74E-03 ± 1.97E-03	5.60E-05 ± 2.24E-05
		25	5.3 ± 1.4	1.03E-02 ± 2.56E-03	1.01E-04 ± 3.22E-05
		35	6.7 ± 2.1	1.35E-02 ± 4.15E-03	1.72E-04 ± 5.83E-05
SK	C	15	2.1 ± 1.5	6.77E-03 ± 4.03E-03	4.33E-05 ± 2.52E-05
		25	2.6 ± 1.2	1.08E-02 ± 4.73E-03	9.55E-05 ± 2.70E-05
		35	4.2 ± 0.7	1.26E-02 ± 2.12E-03	1.73E-04 ± 1.89E-05
ND	NG	15	ND	ND	ND
		25	3.5 ± 0.7	8.68E-03 ± 1.48E-03	8.82E-05 ± 1.33E-05
		35	7.2 ± 2.5	6.72E-03 ± 1.89E-03	1.67E-04 ± 4.48E-05
ND	C	15	2.0 ± 0.5	1.03E-02 ± 2.35E-03	6.04E-05 ± 1.03E-05
		25	4.0 ± 1.4	7.58E-03 ± 2.26E-03	9.88E-05 ± 2.57E-05
		35	4.0 ± 1.1	1.17E-02 ± 2.94E-03	1.35E-04 ± 2.76E-05
CO	NG	15	ND	ND	ND
		25	9.4 ± 2.4	6.88E-03 ± 1.42E-03	1.85E-04 ± 4.46E-05
		35	14.0 ± 2.7	7.05E-03 ± 1.08E-03	3.31E-04 ± 5.38E-05
CO	C	15	ND	ND	ND
		25	3.1 ± 1.0	1.60E-02 ± 5.11E-03	1.91E-04 ± 2.94E-05
		35	7.3 ± 1.3	1.44E-02 ± 2.48E-03	3.52E-04 ± 4.00E-05
TX	NG	15	7.0 ± 2.7	5.43E-03 ± 1.66E-03	5.93E-05 ± 4.35E-05
		25	8.9 ± 2.0	9.02E-03 ± 1.80E-03	1.25E-04 ± 4.59E-05
		35	10.0 ± 1.2	1.38E-02 ± 1.60E-03	3.14E-04 ± 3.85E-05
TX	C	15	6.8 ± 3.9	5.54E-03 ± 2.51E-03	4.66E-05 ± 6.21E-05
		25	6.7 ± 2.8	1.57E-02 ± 6.53E-03	1.86E-04 ± 8.59E-05
		35	8.8 ± 1.4	1.87E-02 ± 2.99E-03	3.17E-04 ± 5.19E-05
CR	NF	15	0.1 ± 0.0	2.89E-01 ± 6.87E-02	4.63E-05 ± 7.20E-06
		25	0.6 ± 0.1	7.64E-02 ± 8.34E-03	6.57E-05 ± 8.07E-06
		35	1.0 ± 0.1	6.22E-02 ± 8.81E-03	1.63E-04 ± 1.41E-05

CR	P	15	0.7 ± 0.4	6.15E-03 ± 2.56E-03	9.63E-06 ± 6.10E-06
		25	0.5 ± 0.1	4.94E-02 ± 1.12E-02	3.96E-05 ± 7.75E-06
		35	3.2 ± 0.3	1.17E-02 ± 9.10E-04	4.83E-05 ± 6.15E-06
BR	NF	15	1.1 ± 0.2	5.61E-02 ± 1.14E-02	8.58E-05 ± 1.90E-05
		25	0.6 ± 0.1	1.11E-01 ± 2.15E-02	2.14E-04 ± 2.11E-05
		35	2.1 ± 0.3	4.92E-02 ± 9.52E-03	3.45E-04 ± 3.46E-05
BR	P	15	1.9 ± 0.3	3.59E-02 ± 6.00E-03	9.64E-05 ± 1.75E-05
		25	3.7 ± 0.7	2.23E-02 ± 4.23E-03	1.37E-04 ± 2.55E-05
		35	6.0 ± 0.7	2.91E-02 ± 3.76E-03	3.35E-04 ± 3.94E-05

612   [†] SK, Saskatchewan; ND, North Dakota; CO, Colorado; TX, Texas; CR, Costa Rica; BR, Brazil

613   [‡] NG, native grassland; NF, native forest; C, cultivated; P, pasture

614   ND values were not determined because the model calculated negative decomposition rates for the slow pool for these samples

615

616   Table 5. Proportions (%) of ion intensity attributable to carbohydrates, peptides, phenols, lignin dimmers, lipids, alkyl-aromatics,

617   various N-containing compounds (VCN), sterols and the remaining unknown m/z fore each of the soils sampled. (mean ± 1 standard

618   deviation, n= 6)

Site[1]	Land use[‡]	m/z 20-56	Carbs	Peptides	Phenols & Lignin Monomers	Lignin dimers	Lipids	Alkyl aromatics	VNC	Sterols	Unknown
SK	NG	52.8	11.0	9.7	4.0	0.3	0.4	2.5	12.9	0.3	4.7
	C	59.0	8.0	8.2	3.0	0.6	0.8	1.9	9.6	0.6	6.6
ND	NG	52.8	11.0	9.1	3.9	0.3	0.5	2.2	13.1	0.3	5.1
	C	54.4	10.1	9.4	4.0	0.3	0.4	2.1	13.1	0.3	4.5
CO	NG	51.8	9.7	8.5	3.9	0.6	0.9	2.5	11.5	0.7	7.5
	C	48.5	8.1	8.4	4.0	1.0	1.8	2.4	9.2	1.1	11.5
TX	NG	53.4	9.1	8.8	3.3	0.6	0.9	2.4	11.0	0.8	7.3
	C	52.1	8.0	7.5	3.5	0.9	1.6	2.2	9.3	1.1	10.7
CR	NF	41.0	15.6	12.3	6.0	0.5	0.2	2.3	14.2	0.4	4.8
	P	47.5	15.4	11.2	4.6	0.3	0.1	2.1	13.3	0.3	3.5
BR	NF	50.0	9.6	9.4	4.2	0.6	0.9	2.1	12.8	0.6	7.5
	P	47.6	11.8	10.3	4.4	0.5	0.7	2.5	13.4	0.6	6.2
Mean		50.9±4.5	10.6±2.6	9.4±1.4	4.1±0.7	0.5±0.2	0.8±0.5	2.3±0.2	11.9±1.8	0.6±0.3	6.7±2.5

619   [†] SK, Saskatchewan; ND, North Dakota; CO, Colorado; TX, Texas; CR, Costa Rica; BR, Brazil

620   [‡] NG, native grassland; NF, native forest; C, cultivated; P, pasture

621   Table 6. Statistically significant m/z values from Martens' uncertainty test and sequential

622   PLS regressions of chemical compositions against respiration rate at two different times

623   of incubation ranked from largest to smallest value of regression coefficient. Where

624   possible, m/z values marked with a compound class identifier based on classifications by

625   Schulten (1996).

626

Day 5	97 (Pep), 98 (CH), 60 (CH), 73 (Pep), 111 (VNC), 58, 87 (Pep), 125 (VNC), 126 (CH), 59 (VNC), 74 (Pep), 123 (VNC), 138 (PLM), 101, 139 (VNC), 165, 75 (Pep)
Day 225	97 (Pep), 98 (CH), 83, 60 (CH), 80, 73 (Pep), 111 (VNC), 58, 125 (VNC), 87 (Pep), 126 (CH), 59 (VNC), 74 (Pep), 138 (PLM), 123 (VNC), 86, 72 (CH), 101, 139 (VNC), 168 (PLM), 143, 165, 428, 194 (PLM), 485, 479, 387, 446, 385, 373, 415, 332, 459

627   CH = carbohydrate; Pep = peptide; PLM = phenol and lignin monomer; LD = lignin

628   dimer; LP = lipid, alkane, alkene, fatty acid and n-alkyl ester; AA = alkyl aromatic; VNC

629   = various nitrogen containing compounds; S = sterol

630

631   **Figure Legend**

632   Figure 1. Decomposition rates over time at 15ºC, 25ºC, and 35ºC for the twelve soils. NG

633   is native grassland and NF is native forest. Values represent means ± SEM (n = 4).

634

635   Figure 2. Correlation between the C to N ratio of the soil and the size of the active

636   fraction in percent of total SOC for the twelve soils sampled. All sites are represented by

637   closed circles except Colorado cultivated (CO-C), an unfilled square, and Brazil native

638   forest (BR-NF), an unfilled triangle.

639

640   Figure 4. Normalized mass spectrum for spectral range m/z=57-500 from the pyrolysis

641   molecular beam mass spectrometry analysis of Saskatchewan native grassland (NG), N.

642   Dakota cultivated, and the difference between the two spectra. In the difference graph

643   m/z that are more abundant in the native grassland are positive and m/z that are more

644   abundant in the cultivated are negative. Spectra are means of 6 samples.

645

646   Figure 3. PCA scores of whole-soil samples analyzed by py-MBMS for the twelve soils

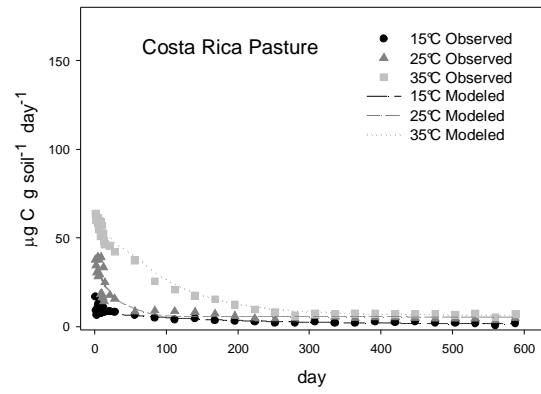647   sampled. Closed symbols are native treatments, open symbols are cultivated or pasture

648   treatments.
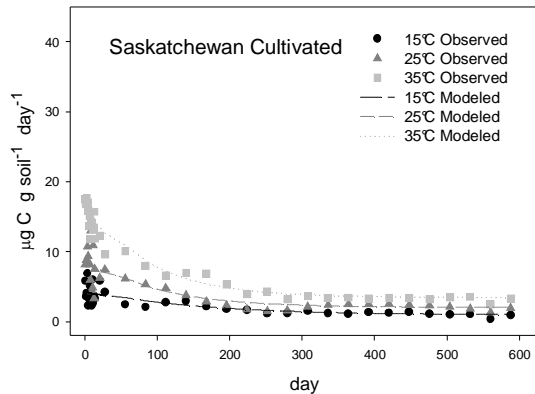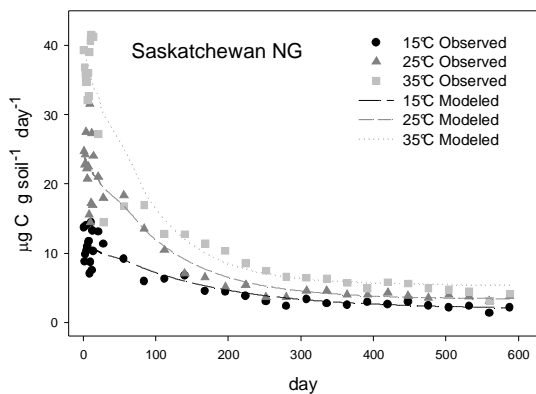
649
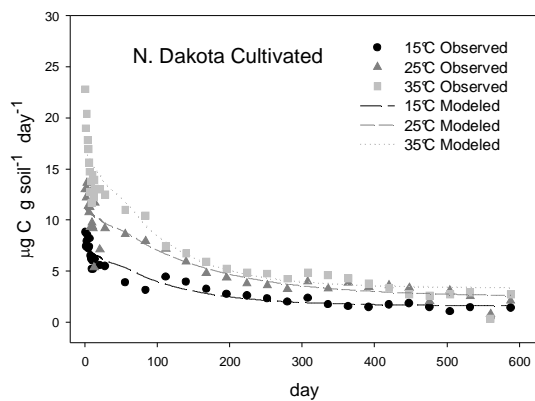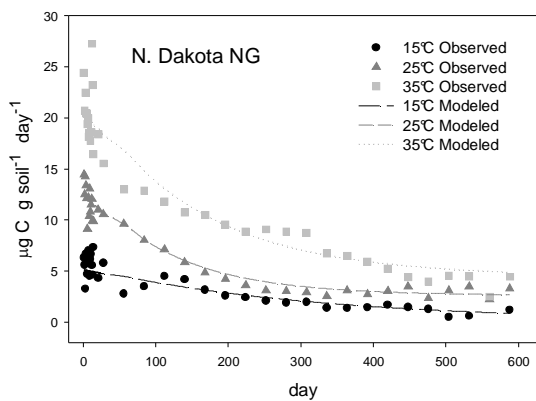
650
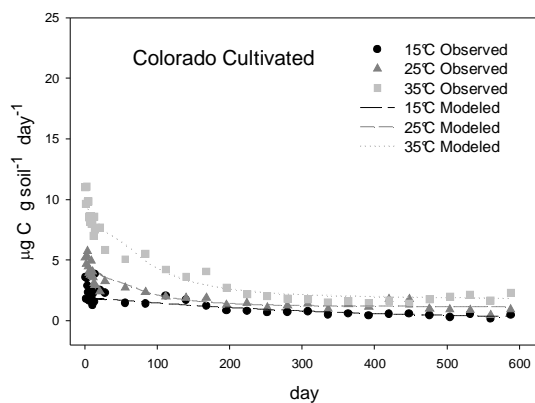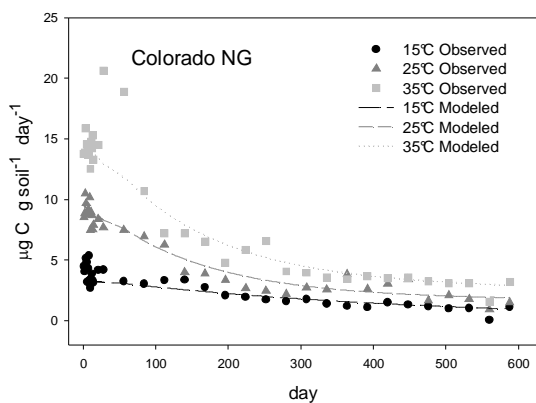
651

652

653

654

655   Figure 1.

Without CO-C and BR-F
y = 21.95 + (-1.65 x C:N)
r^2 = 0.84

With all samples
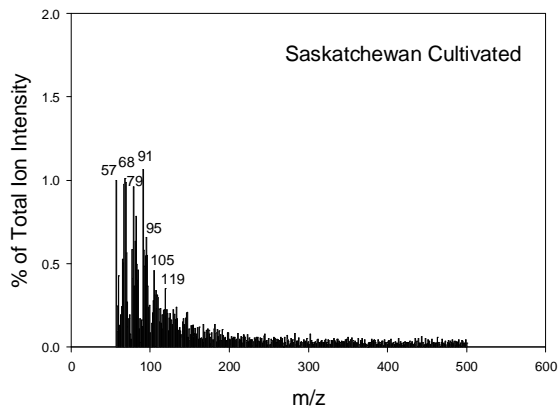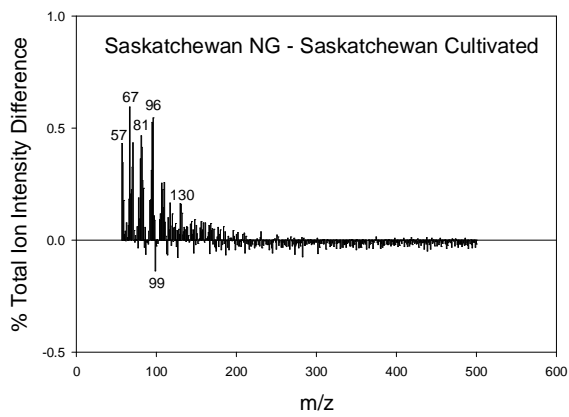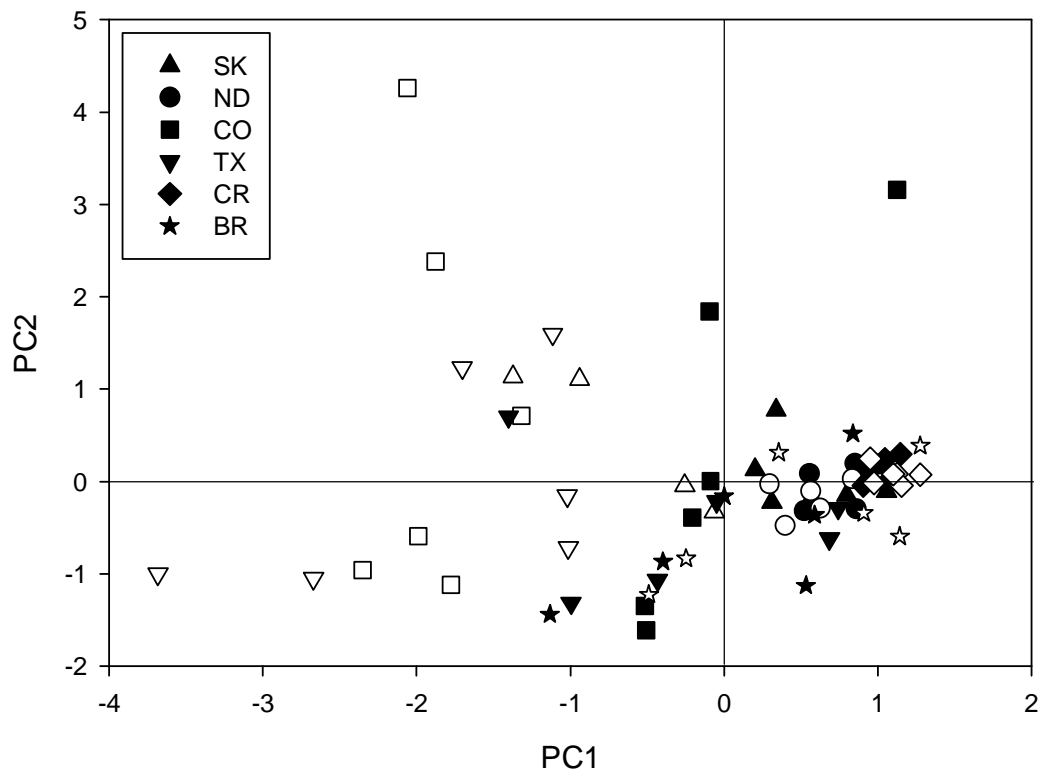y = 13.72 + (-0.95 x C:N)
r^2 = 0.34
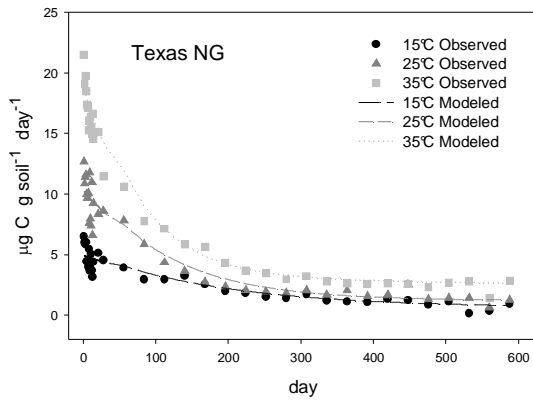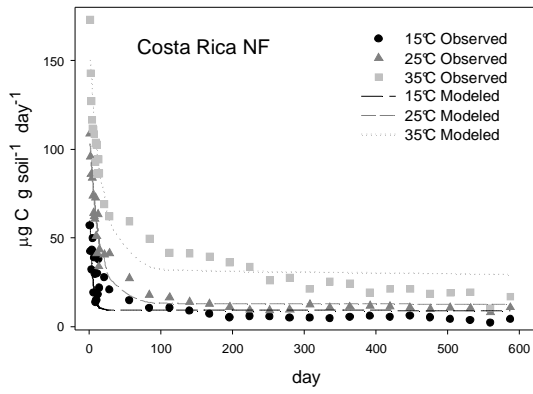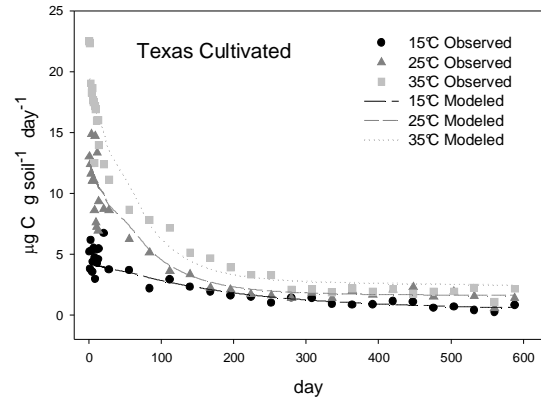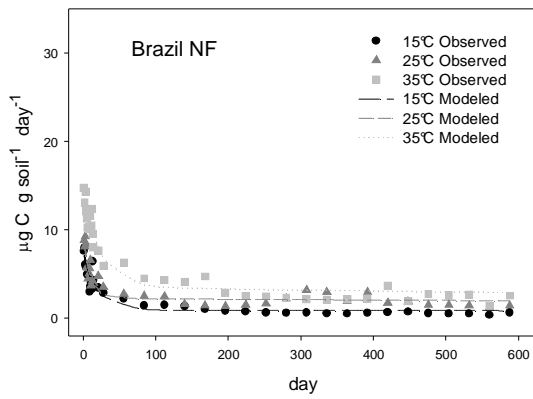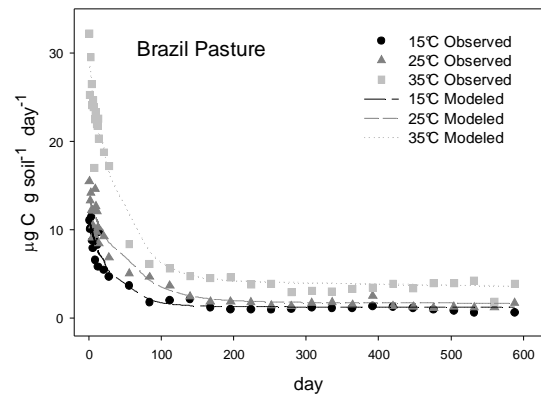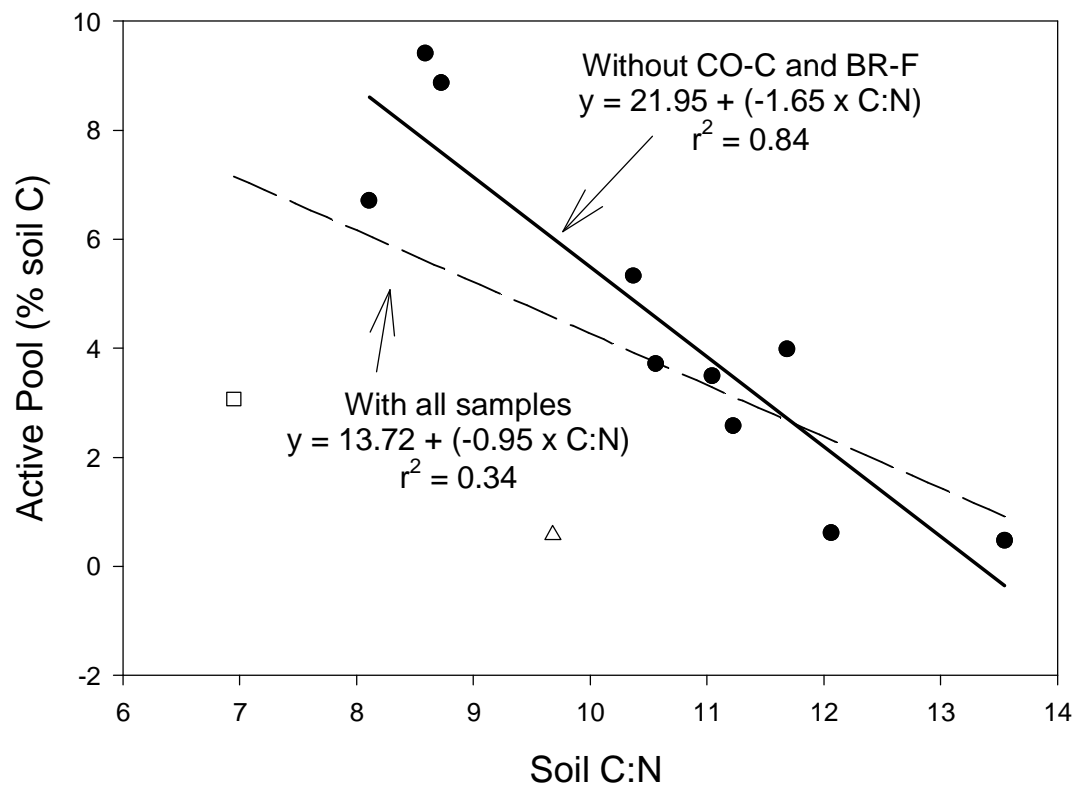
656

657   Figure 2.

658

41
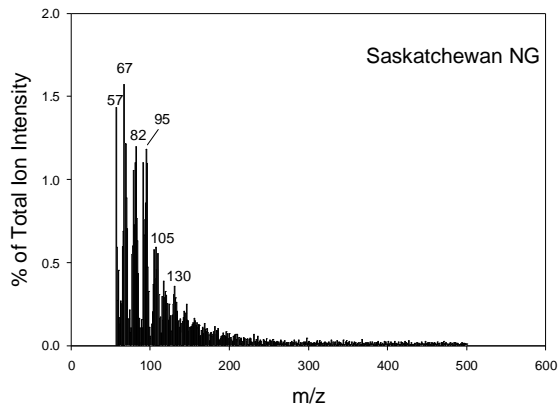
659

660

661

662   Figure 3.

663

664   Figure 4.