NERC Open Research Archive
*sharing our research*

Centre for Ecology & Hydrology
NATURAL ENVIRONMENT RESEARCH COUNCIL

# Article (refereed) - postprint

Contact CEH NORA team at
noraceh@ceh.ac.uk

# Climate change effects on the stability and chemistry of soil organic carbon pools in a subalpine grassland

Jérémy Puissant[a,b], Robert TE Mills[c,d,h], Bjorn JM Robroek[c,d], Konstantin Gavazov[e], Yves Perrette[f], Sébastien De Danieli[a], Thomas Spiegelberger[a], Alexandre Buttler[c,d,g], Jean-Jacques Brun[a], Lauric Cécillon[a]

[a]Université Grenoble Alpes, Irstea, UR EMGR, 2 rue de la Papeterie-BP 76, F-38402 St-Martin-d'Hères, France
[b]Centre for Ecology and Hydrology, Maclean Building, Benson Lane, Crowmarsh Gifford, Wallingford, Oxfordshire OX10 8BB, UK
[c]Ecole Polytechnique Fédérale de Lausanne EPFL, School of Architecture, Civil and Environmental Engineering ENAC, Laboratory of ecological systems ECOS, Station 2, 1015 Lausanne, Switzerland
[d]Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Site Lausanne, Station 2, 1015 Lausanne, Switzerland.
[e]Climate Impacts Research Centre, Department of Ecology and Environmental Science, Umeå University, 98107 Abisko, Sweden
[f]EDYTEM Environnements, DYnamiques et TErritoires de la Montagne, Université de Savoie Laboratoire EDYTEM - UMR5204, Bâtiment « Pôle Montagne », F-73376 Le Bourget du Lac cedex
[g]Laboratoire de Chrono-Environnement, UMR CNRS 6249, UFR des Sciences et Techniques, 16 route de Gray, Université de Franche-Comté, F-25030 Besançon, France
[h]Lancaster Environment Centre, Lancaster University, Lancashire, UK, LA1 4YQ

Corresponding author: Tel.: +44 1491692547; E-mail address: jeremy.puissant@gmail.com

**Abstract**

Mountain soils stock large quantities of carbon as particulate organic matter (POM) that may be highly vulnerable to climate change. To explore potential shifts in soil organic matter (SOM) form and stability under climate change (warming and reduced precipitations), we studied the dynamics of SOM pools of a mountain grassland in the Swiss Jura as part of a climate manipulation experiment. The climate manipulation (elevational soil transplantation) was set up in October 2009 and simulated two realistic climate change scenarios. After four years of manipulation, we performed SOM physical fractionation to extract SOM fractions corresponding to specific turnover rates, in winter and in summer. Soil organic matter fraction chemistry was studied with ultraviolet, 3D fluorescence, and mid-infrared spectroscopies. The most labile SOM fractions showed high intra-annual dynamics (amounts and chemistry) mediated via the seasonal changes of fresh plant debris inputs and confirming their high contribution to the microbial loop. Our climate change manipulation modified the chemical differences between free and intra-aggregate organic matter, suggesting a modification of soil macro-aggregates dynamics. Interestingly, the four-year climate manipulation affected directly the SOM dynamics, with a decrease in organic C bulk soil content, resulting from significant C-losses in the mineral-associated SOM fraction (MAOM), the most stable form of SOM. This SOC decrease was associated with a decrease in clay content, above- and belowground plants biomass, soil microbial biomass and activity. The combination of these climate changes effects on the plant-soil system could have led to increase C-losses from the MAOM fraction through clay-SOM washing out and DOC leaching in this subalpine grassland.

**Keywords:** Water extractable organic carbon; Particulate organic matter; Mineral associated organic matter; Infrared spectroscopy; 3D Fluorescence spectroscopy

1. **Introduction**

The fate of soil organic matter (SOM), in terms of accumulation and decomposition is an issue of concern, since a small modification of its global stock may alter the atmospheric greenhouse gas concentration at a decadal timescale (Eglin et al., 2010). Several specific, simultaneously operating, mechanisms in soils can protect SOM from microbial decomposition and thus preserve SOM compounds that would otherwise be rapidly degraded (Leifeld et al., 2009; Sollins et al., 2007; Torn et al., 2009; von Lützow et al., 2006): (i) the selective preservation of recalcitrant materials, a key process during the early phase of the decomposition process (i.e. months to years); (ii) the spatial inaccessibility of SOM compounds (e.g. soil aggregate formation, hydrophobicity of SOM compounds), (iii) the adsorption/co-precipitation of SOM on/by mineral surfaces and (iv) pedoclimatic conditions detrimental to SOM mineralization (e.g., soil frost, low soil pH). Depending on the stabilization mechanisms involved, SOM compounds can remain in soils from days to millennia, and can therefore be roughly classified into SOM pools with contrasting turnover rates (Baisden et al., 2002; Leifeld et al., 2009).

Many physical and/or chemical SOM fractionation methods have been developed to isolate C pools with contrasting turnover rates (von Lützow et al., 2007). The combination of size and density separation is one of the best methods available to obtain such SOM fractions with specific residence times (Sohi et al., 2001; von Lützow et al., 2007) such as: (i) the water extractable organic C (WEOC, <0.45μm); (ii) the free particulate organic matter (freePOM); (iii) the occluded particulate organic matter (occPOM) and (iv) the mineral associated organic matter (MAOM) fractions. The WEOC fraction could be viewed as a readily available resource for microbial communities (Marschner and Kalbitz, 2003). Although soil WEOC only accounts for a few percent of SOC (ca. 0.05-2%, see the review from von Lützow et al., 2007), some components of this fraction cycle extremely rapidly (ca. 4000 times a year,

83   Boddy et al., 2007) underlining its key functional role in soil. The labile particulate organic

84   matter (POM) fraction consists mainly of weakly decomposed plants debris (Poirier et al.,

85   2005; von Lützow et al., 2007). Particulate SOM fractions could be protected within soil

86   aggregates resulting to a change in its chemistry and its turnover rate. Consequently, the

87   freePOM has a chemistry closer to the one of plant biomass than the occPOM (Helfrich et al.,

88   2006; Kölbl and Kögel-Knabner, 2004; Poirier et al., 2005) and a higher turnover rate (e.g. 6

89   and 22 years for freePOM compared to 28 and 83 for occPOM according to John et al., 2005

90   and Baisden et al., 2002). In cold areas, climate and low pH conditions temporarily stabilize

91   these labile POM fractions. The mean turnover rate of the freePOM fraction was recently

92   estimated within the range 80-90 years in various mountain grassland soils (Budge et al.,

93   2011; Leifeld et al., 2009, Meyer et al, 2012a), representing from 20 to 80 % of total C stocks

94   in the first 10 cm of mineral soils (Leifeld and Kögel-Knabner, 2005; Leifeld et al., 2009;

95   Saenger et al., 2015). The mineral associated organic matter (MAOM) fraction represents the

96   passive/slow cycling C pool, with a low turnover rate (142-250 years in Meyer et al, 2011;

97   534 years in Budge et al, 2011) and a highly microbially processed SOM (low C:N ratio

98   closer to the one of microbial biomass than to the one of plant biomass, and high NMR alkyl-

99   C/O-alkyl-C ratio; Baisden et al., 2002; Budge et al., 2011).

100        Mountain regions are currently experiencing strong climatic changes altering the

101   temperatures, precipitation and the intensity and duration of seasons. Indeed, shorter periods

102   of snow cover, resulting in longer growing seasons, have been reported to coincide with

103   increasing occurrences of summer droughts and winter freeze-thaw cycles in soils (Gobiet et

104   al., 2014). Such climate changes are likely to alter the C dynamics of mountain soils, from the

105   modification of plant C inputs to the alteration of the various mechanisms of SOM protection

106   from microbial decomposition (Groffman et al., 2001; Davidson and Janssens, 2006; Conant

107   et al, 2011). More specifically, the high C stocks of mountain soils, with high proportions of

4

108 freePOM C protected by current cold climate conditions may be highly vulnerable to climate

109 changes (Sjögersten et al., 2011; Torn et al., 2009). Soil moisture conditions are known to

110 alter the WEOC SOM fraction, especially when soil dry conditions are followed by rapid

111 rewetting (Toberman et al., 2008; Zsolnay, 2003). However a marked gap in the

112 understanding of the WEOC fraction dynamics in soils remains (Embacher et al., 2007). The

113 respective amounts of freePOM and occPOM fractions are mediated by C inputs from plants

114 and by the formation/deformation of soil aggregates. Soil aggregation depends on physical

115 processes such as freeze/thaw and dry/rewetting cycles, as well as biological processes such

116 as the activity of soil fauna and plant roots (Six et al., 2004; Davidson et Janssens., 2006;

117 Erktan et al., 2016) that show a strong seasonal pattern in mountain regions and may be

118 altered by climate changes. However the effects of climate modifications on soil aggregation

119 processes and their consequences on the dynamics of SOM fractions have not been studied in

120 great depth (e.g. Cécillon et al., 2010; Conant et al, 2011). The MAOM fraction response to

121 climate change is also highly uncertain and could depend on the type of chemical association

122 of organic C with minerals (Conant et al, 2011). However, the absolute C-losses from this

123 passive/slow cycling C pool under climate changes are expected to be lower than those from

124 the particulate SOM fractions in mountain soils (Sjögersten et al., 2011).

125     In this study, we investigated the effects of a climate change experiment on the

126 dynamics of SOM pools in subalpine grasslands from the Swiss Jura. We focused on the size

127 and chemistry of four SOM fractions (WEOC, freePOM, occPOM and MAOM fractions) at

128 two sampling dates representing two contrasting seasons: winter and summer. We performed

129 a climate manipulation experiment by transplanting grassland mesocosms along an altitudinal

130 gradient. This four-year climate experiment simulated two climate change scenarios close to

131 current predictions for the 21th century with increased air temperatures ranging between 2 °C

5

132   and 4 °C and decreased precipitation ranging between 20 % and 40 % (C2SM, 2011; Frei et

133   al., 2006; Meehl et al., 2007).

134      We hypothesized that (i) the POM-C fractions (freePOM + occPOM) would represent

135   a large proportion of total SOC in this historically grazed subalpine grassland, especially in

136   the control plots not affected by the climate manipulation ; (ii) the climate manipulation and

137   the strong seasonal climatic changes would affect the quantity and the chemistry of labile

138   SOM fractions (WEOC, free-POM and occPOM). The climate manipulation may affect labile

139   SOM fractions proportionally to their mean turnover rate: WEOC>freePOM>occPOM. (iii)

140   the climate change manipulation would not affect strongly the most stable C pool, stabilized

141   by mineral interactions (MAOM fraction).

142

143      2.   **Materials and methods**

144

145      2.1. Study site, experimental manipulations, and seasonal sampling

146   The experiment was located in the Swiss Jura mountain range and consisted of a high-to-low

147   elevation soil translocation that, with respect to a disturbance control, simulates a climate

148   warming with an average temperature increase of +2 °C and +4 °C and a precipitation

149   decrease of 20 % and 40 % at the intermediate site and at the lowest the site, respectively.

150   Details about the experimental set-up can be found in Gavazov (2013) and Gavazov (2014a

151   and 2014b). Briefly, in 2009 mesocosm containing turf monoliths of undisturbed soil (30 cm

152   depth) and vegetation, were randomly taken from a mesic grassland grazed by cattle located at

153   1350 m a.s.l. and transplanted to (i) a control site (1350 m a.s.l., Les Amburnex, N 46°54′, E

154   6°23′); (ii) an intermediate site (1010 m a.s.l., Saint-George, N 46°52′, E 6°26′) and to (iii) a

155   low-elevation site (570 m a.s.l., Arboretum d'Aubonne, N 46°51′, E 6°37′). Five replicated

156    mesocosms were transplanted per site. The main vegetation consisted of graminoids with few

157    forbs, and the soil could be classified as a Hypereutric Cambisol (IUSS Working Group

158    WRB, 2014) on Jurassic limestone. The aboveground biomass was removed each summer

159    simulating grazing of the pasture to avoid confounding effect of abandonment. During the

160    fourth year of the soil transplantation experiment, two sampling campaigns were performed in

161    winter and summer (February 20th 2013, September 2nd 2013, respectively). Five intact cores

162    of topsoil (5 cm diameter × 10 cm length) per site (one core per mesocosm) were taken at

163    each sampling date (Puissant et al., 2015). Moreover, an additional sampling campaign were

164    performed in summer 2014 to evaluate the soil carbon content along the entire depth of the

165    mesocosm. Five intact soil cores (5 cm diameter × 30 cm length) per site (one core per

166    mesocosm) were taken at the summer 2014 sampling date (Supplementary Fig.S1).

167

168        2.2. Soil microclimate

169    Soil temperature and soil volumetric water content were monitored in all mesocosms over the

170    four-year experimental period (Fig.1). Details of the soil microclimate conditions at the two

171    sampling dates corresponding to winter 2013 and summer 2013 season can be found in

172    Puissant et al. (2015). Briefly, the winter soil temperature was ca. 1 °C at all sites and soil

173    moisture (0-10cm) was ca. 45%. Conversely, soil temperature and moisture showed

174    significant differences between transplantation sites in summer, with a daily mean

175    temperature of 13.2 °C, 16.1 °C, 18.4 °C and a volumetric water content of 33 %, 26 % and

176    21 % for the control, the intermediate and the lowest sites, respectively, for the summer

177    periods of 2009 to 2013. Overall, our climate manipulation increased the mean annual soil

178    temperature by 2 °C and 4 °C (November 2012 to October 2013) at the intermediate (1010m)

179    and at the lowest (570m) sites, respectively. Soil moisture showed a clear decrease throughout

180    the year at the lowest site and a more complex pattern at the intermediate site, depending on

181    the season. Furthermore, the climate manipulation resulted in a longer and warmer plant

182    growing season (with a decrease in snow cover duration) at the intermediate and the lowest

183    sites (Fig.1; Puissant et al., 2015).

184

185          2.3. Basic characterization of bulk soil samples

186    Gravimetric soil water content was measured by drying soil at 105 °C for 48 h according to

187    NF ISO 16586 (2003). For all chemical and texture analyses of bulk topsoils (0-10 cm),

188    samples were dried at 40 °C and sieved (2 mm) following NF ISO 11464 (2006). The particle

189    size distribution (i.e. soil texture) was determined by wet sieving and sedimentation using the

190    Robinson pipette method, according to NF X31-107. Soil pH was measured in $H_2O$ (1:5

191    vol:vol) according to the protocol NF ISO 10390 (2005). Calcareous content was determined

192    following the norm NF ISO 10693 (1995). Soil cations exchange capacity (CEC) was

193    determined according to Metson method described in the protocol NF X 31-130 (1999).

194    Organic C and total nitrogen (N) concentrations were measured by the Dumas dry combustion

195    method after decarbonation (NF ISO 10694, 1995; and 13878, 1995, respectively).

196

197          2.4. Water-extractable organic C fraction

198    To obtain the WEOC fraction, 40 mL of deionized water was added to 10 g of moist sieved (2

199    mm) soil, and shaken for 20 minutes at 250 rpm. Samples were then centrifuged at 10,000 $g$

200    for 10 minutes, after which the solution was filtered through 0.45 mm Millipore filter and

201    immediately stored at -20 °C until analysis. Soil WEOC content was measured using a total

202    organic carbon analyzer (Shimadzu Inc., Kyoto, Japan). The analyzer was calibrated for total

203    dissolved C (TDC) and dissolved inorganic C (DIC) using a calibration solution of potassium

204    hydrogen phthalate ($C_8H_5KO_4$) and a solution containing a mixture of sodium hydrogen

205       carbonate (NaHCO$_3$) and sodium carbonate (Na$_2$CO$_3$) for TDC and DIC respectively. WEOC

206       was calculated as the difference between TDC and DIC and expressed in mg C.g$^{-1}$ soil.

207

208           2.5. Soil organic matter density fractionation

209       Three SOM fractions (freePOM, OccPOM and MAOM) were separated by density

210       fractionation of oven dried (40 °C) and sieved (< 2 mm) soil samples following Leifeld et al.

211       (2005, 2009). Briefly, 15 g of soil were placed into a 50 mL centrifuge tube. A sodium

212       polytungstate solution (density = 1.6 g cm$^{-3}$) was added up to the 50 mL line and the tube was

213       gently inverted several times. After 2 hours, floating materials (<1.6 g cm$^{-3}$) corresponding to

214       the freePOM fraction, were collected and washed thoroughly with deionized water through

215       0.45 μm nitrocellulose membrane filters. This first step was repeated four times to obtain all

216       remaining freePOM. Then the remaining pellet was re-suspended in sodium polytungstate and

217       treated with ultra-sonication (22 J mL$^{-1}$ in an ice bath using a Branson 250 calibrated

218       according to Schmidt et al (1999) so as to breakdown all soil macro-aggregates (Leifeld and

219       Kögel-Knabner, 2005). After sonication, samples were centrifuged at 10,000 g for 10 minutes

220       and floating materials (occPOM fraction) were collected and washed thoroughly with

221       deionized water through 0.45 μm nitrocellulose membrane filters. This step was repeated four

222       times to collect all occPOM released by the sonication treatment. The remaining pellet

223       corresponding to the MAOM fraction was centrifuged, re-suspended and washed with

224       deionized water through 0.45 μm nitrocellulose membrane filters several times, until

225       obtaining an electrical conductivity < 0.50 mS cm$^{-1}$ (Leifeld and Kögel-Knabner, 2005; Meyer

226       et al., 2012a). We used 0.45 μm nitrocellulose membrane filters so as to characterize the SOC

227       fraction until the WEOC size definition. All washed fractions were oven dried at 40 °C and

228       weighed. Organic C and total N concentrations of the freePOM, occPOM and MAOM

229       fractions were determined using the same methods as for bulk soil samples (see section 2.3).

230   Organic C and total N concentrations of SOM fractions (expressed as g C or N kg$^{-1}$ SOM

231   fraction) were then expressed as percent of the SOC and total N contents of bulk soil samples

232   (i.e. SOC and total N distribution in SOM fractions).

233

234   2.6. Chemistry of the soil organic matter fractions

235   2.6.1. Chemistry of the WEOC fraction

236   The chemistry of the WEOC fraction was qualitatively assessed using ultraviolet (UV)

237   spectroscopy. The absorbance of the WEOC fraction at 280 nm was used as an indicator of its

238   aromaticity (Kalbitz et al., 2003). Three dimensions (3D) spectrofluorescence measurements

239   were then performed on the WEOC fraction with a spectrofluorometer (Varian Cary-Eclipse).

240   Placed in a quartz cell (10x10 mm – Hellma Analytics), fluorescence emissions of solutions

241   were recorded between 250 and 500 nm with 5-nm slits (1 nm step) and were excited in the

242   range from 220 to 310 nm with a step of 15 nm. In order to overcome inner filter effects,

243   solutions were diluted until absorbance at 256 was inferior to 0.01. All data were expressed in

244   Quinine Sulfate Units (Fluorescence data were used to characterise the main fluorophore

245   compounds and their distribution in the WEOC). In order to determine the fluorescent

246   protein-like moieties, emission spectra were simulated by the linear combination of log-

247   normal functions which were shown to simulate organic matter fluorescence (Siano and

248   Metzler, 1969). Simulations were performed by a constraint (for center location and width)

249   least square simulation of a set of user-defined bands (number and positions) using a matlab

250   optimisation toolbox. This method is effective in simulating protein-like fluorophores whose

251   emissions are often masked by Rayleigh or Raman scatterings. The bands were ascribed to

252   Tyrosine-like ($\lambda$exc/$\lambda$emi: 235/310), tryptophane-like (235;295 / 350), fulvic-like

253   (235;295;310 /410) and humic-like (295; 310 /480) compounds.

254

255        2.6.2. Chemistry of the POM fractions

256    The chemistry of the POM fractions (freePOM and occPOM) was assessed using mid-infrared

257    (MIR) spectroscopy. Prior to these analyses, POM fractions were ball-milled (< 0.25 mm

258    using a Retsch ZM 200) and further dried overnight at 40 °C to limit interferences with water,

259    without altering OM chemistry. Crushed samples were analyzed using a Nicolet iS10 FT-IR

260    spectrometer (Thermo Fisher Scientific Inc., Madison, WI, USA). Spectral acquisition was

261    performed by diamond attenuated total reflectance (MIR-ATR) spectroscopy over the spectral

262    range 4,000–650 $cm^{-1}$, with spectral resolution of 4 $cm^{-1}$ and 16 scans per replicate (2

263    replicates per sample). All MIR-ATR spectra were corrected for atmospheric interferences

264    ($H_2O$ and $CO_2$). Spectral data were further processed and analyzed using the hyperSpec

265    (Beleites and Sergo, 2011), signal (signal developers, 2013) and ptw (Bloemberg et al 2010)

266    packages in the R environment, software version 2.14.0 (R Development Core Team 2011).

267    Five spectral regions corresponding to specific C functional groups were chosen for further

268    characterization (1) the saturated hydrocarbons (SAT) region 2,750–3,025 $cm^{-1}$ corresponding

269    to alkyl C ($CH_2$, $CH_3$, and CH); (2) the unsaturated (UNSAT) region 1,700–1,760 $cm^{-1}$ and

270    1,576–1,618 $cm^{-1}$ corresponding to C=O and aromatic C=C bonds respectively; (3) the

271    polysaccharide (POLY) region 1,222–1,287 $cm^{-1}$ corresponding to O-Alkyl C, and (4) the

272    aromatic CH (AROCH) region 852–898 $cm^{-1}$ (Supplementary Fig.S2) . The contribution of

273    minerals (e.g. phyllosilicates) or polytungstate pollution to the intensities of the five

274    waveband-regions was negligible for these POM fractions. The selected wavebands were used

275    to calculate seven proxies of POM chemistry, according to Pengerud et al (2013) and Robroek

276    et al. (2015): (1) SAT/SOC = hydrophobicity index (SAT = saturated hydrocarbons = Alkyl

277    C, and SOC = soil organic carbon content); (2) C=O/SOC (index of oxidation); (3) C=C/SOC

278    (aromaticity index); (4) MI = C=C/UNSAT (maturation index) (5) POLY/SOC

279    (polysaccharide content); (6) AROCH/C=C (condensation index = degree of condensation of

280   aromatics), (7) SAT/POLY to estimate the Alkyl-C/Alkyl-O which is used as an indicator of

281   microbial transformation (Budge et al, 2011).

282

283       2.7. Statistical analyses

284   The effect of climate conditions (soil transplantation and sampling dates) and the effect of

285   SOM pools (i.e. SOM fractions) on SOM chemistry (UV and FLUO and MIR-ATR

286   spectroscopy indices), were assessed by three-way mixed effects ANOVAs (Supplementary

287   Table 1). Fixed factors were the sampling date, the transplantation site and the SOM fraction,

288   while the actual mesocosm was added as a random factor and the sampled core (from which

289   the SOM fraction originates) as a nested factor. Data was log or square root transformed when

290   necessary to respect assumptions of normality and homoscedasticity of the residuals. To test

291   the effect of sampling date (season) on each SOM fraction separately, one-way mixed

292   ANOVAs was performed at each transplantation site with the sampling date as fixed factors

293   and the actual mesocosm as a random factor. To test the effect of the climate change

294   experiment (site effect) on each individual variable (SOM fraction, pH, soil moisture, etc.),

295   one-way ANOVA was performed at each season (winter and summer). When significant

296   ANOVAs were obtained at the 5 % error probability threshold, a Tukey's post-hoc test was

297   applied. All statistical analyses were performed under the R environment software 2.14.0 (R

298   Development Core Team 2011), using the R package NLME (Pinheiro et al., 2014).

299

300   3.  **Results**

301

302       3.1. Basic soil characteristics

303   All soils from the transplanted mesocosms were determined as clay textured soils (USDA

304   texture triangle). An effect of the transplantation on topsoil (0-10 cm) texture was observed,

305  manifested by a significant decrease in clay content at the intermediate site (clay content =

306  40.2 % at 1010 m a.s.l.) compared to the control site (clay content = 52.6 % at 1350 m a.s.l.;

307  p-value = 0.02; Table 1). Clay content at the lowest site (at 570 m a.s.l.) was not significantly

308  different from neither the control site nor the intermediate site (clay content = 44.5 %; p-value

309  = 0.12 and 0.52 respectively; Table 1). SOC content of bulk topsoil showed a similar response

310  to the transplantation, with a significant decrease at the intermediate site in winter and at the

311  lowest and intermediate sites in summer (Table 1). This decrease in SOC content induced by

312  the climate change experiment was confirmed at the summer 2014 sampling date. Whatever

313  the depth sampled, the same trend was observed with a decrease in SOC content at the two

314  transplanted site. The difference in SOC content was not significant in 0-5 cm and in 5-10 cm

315  soil layers but were significant for the intermediate site in 10-15 cm soil layer and for both

316  transplanted sites in 15-30 cm soil layer. This result confirmed the decrease in SOC content

317  and showed that the decrease has been accentuated with increasing the soil depth. Surface

318  SOC content showed a strong correlation with clay content (r = 0.67, p-value = 0.006). We

319  did not observe any effect of transplantation on soil pH and CEC (Table 1). A seasonal effect

320  on CEC was observed for the lowest site (570 m a.s.l.) with a lower value in summer (Table

321  1).

322

323  3.2. Distribution and chemistry of organic C in SOM fractions

324  3.2.1. Water extractable organic carbon

325  Climate manipulation did not impact the WEOC content between the sites (Fig.2). In the two

326  recipient sites (570 and 1010 m) WEOC was lower in winter as compared to summer (Fig.2).

327  Regarding chemistry of organic C in the WEOC fraction, its aromaticity measured by UV

328  absorption at 280 nm was significantly higher in summer than in winter, irrespective of site.

329  This seasonal effect on WEOC chemistry was confirmed by many 3D fluorescence indices.

13

330    Indeed, WEOC was significantly richer in fulvic-like and humic-like compounds compared to

331    tyrosine-like compounds at the summer sampling date. No seasonal change in

332    tryptophan/humic or tryptophan/fulvic indices was observed (Fig.2). Finally the fluorescence

333    ratio WEOC humic/fulvic increased in summer while the ratio tyrosine/tryptophan increased

334    in winter. We observed a significant decrease in the WEOC tryptophan/fulvic fluorescence

335    ratio (in summer and winter) and in the WEOC tyrosine/fulvic fluorescence ratio (summer

336    only) in the lower altitudinal sites, as compared to the control site (Fig.2).

337

338        3.2.2. SOM density fractions

339    The SOM fractionation scheme used in this study was rather conservative, with an average of

340    only 4.7 % losses of total SOC content when summing the organic C contribution of each

341    density fraction. The mean annual distribution of SOC in SOM fractions showed that most

342    SOC belonged to the MAOM fraction (86 % of SOC content) while freePOM and occPOM

343    comprised 6 % and 8 % of total SOC content respectively.  WEOC contribution to SOC was

344    very small (i.e. 0.2%).

345

346        3.2.2.1. Free particulate organic matter (freePOM)

347        Even though the organic C content of the freePOM fraction increased significantly in

348    winter compared to the summer sampling date (significant for the control site; Table 2), its

349    contribution to bulk topsoil was greater at the summer sampling date (10.1%) than at the

350    winter sampling date (1.9%) for all sites, representing ca. 6.9 gC kg$^{-1}$ dry soil in summer and

351    1.2 gC kg$^{-1}$ dry soil in winter (Fig.3). Under the strongest climate change scenario

352    (transplantation site at 570 m a.s.l.) and at the summer sampling date, the freePOM organic C

353    contribution to SOC bulk content was significantly higher than the two other sites (1010 m

354    and 1350 m a.s.l.). The chemistry of the freePOM fraction was not impacted by the climate

14

manipulation except for the C=O/SOC oxidation index which was significantly lower in

winter at the lowest site compared to the control site (Table 2). Conversely, the sampling date

(winter vs. summer) strongly affected the chemistry of the freePOM fraction, which was less

oxidized (C=O/SOC) and condensed (CI), more aromatic and matured (MI index) in summer

than in winter. This significant effect of the sampling date was generally observed at the

control site but not at the two other transplantation sites, even if the trend was similar (Table

2). The Alkyl-C/Alkyl-O ratio of the freePOM, which can be used as an indicator of its degree

of microbial transformation (Baldock et al., 2007; Budge et al 2011), was consistently higher

in winter than in summer despite that this trend was significant only for the intermediate site

(1010 m a.s.l.).

### 3.2.2.2. Occluded particulate organic matter (OccPOM)

Contrary to the freePOM, the contribution of the occPOM organic C to the SOC bulk content

(5.5 gC kg$^{-1}$ dry soil, i.e. 8%) of topsoils was neither impacted by seasons nor by soil

transplantation (climate manipulation; Fig.3). A strong sampling date effect on occPOM

organic C content was observed whatever the site considered with a more organic C

concentrated occPOM in winter than in summer (Table 2).

The chemistry of the occPOM fraction was not impacted by the climate manipulation.

Conversely, we observed a significant effect of the sampling date on the chemistry of the

occPOM fraction. The organic C of the occPOM fraction was less humified (HI), had a lower

PI index (polysaccharide), and its aromatic C functional groups were less condensed (CI) at

the winter sampling date than at the summer sampling date (Table 2). In contrast to the

freePOM, the C-alkyl/O-Alkyl ratio of the occPOM fraction was higher at the summer

sampling date than at the winter sampling date, even if this trend was significant only for the

lowest site (570 m a.s.l.). Moreover, at the summer sampling date the C-alkyl/O-Alkyl ratio

380    was lower in the freePOM than in the occPOM fraction (significant for the two lowest sites,

381    Table 2). The C/N ratio was higher in freePOM than in occPOM at both sampling dates even

382    if this difference was relatively low and only significant at the summer sampling of the

383    intermediate site (Table 1). We observed an interaction for some IR indices between sites and

384    the chemical differences composition of the fractions. Indeed, the chemical differences

385    between freePOM and occPOM fraction were modulated by the climate change experiment

386    (i.e. site versus fraction effect; Supplementary Table 1).

387

388        3.2.2.3. Mineral associated organic matter (MAOM)

389        We observed a decrease in the MAOM organic C content at the intermediate (1010 m

390    a.s.l., significant in summer and winter) and the lowest site (570 m a.s.l., significant only in

391    summer) compared with the control site (1350 m a.s.l.; Fig.3). The bulk organic chemistry of

392    the MAOM fraction, assessed with the C:N ratio, strongly differed from the chemistry of the

393    freePOM and the occPOM fractions, with a decreasing average C:N ratio from the freePOM

394    (19.3), occPOM (18), to the MAOM (10) fraction. Moreover the SOC content of the MAOM

395    fraction were significantly affected by the climate change manipulation but not by the

396    seasonal change. Indeed, the mean SOC content of the MAOM fraction was significantly

397    lower at the lowest site ($59 \pm 4$ g kg$^{-1}$, p-value =0.003) and at the intermediate site ($52 \pm 4$ g

398    kg$^{-1}$, p-value <0.001) compare to the control site ($70 \pm 2$ g kg$^{-1}$).

399

400    4. **Discussion**

401

402        The majority of the SOC were found in the MAOM-C fraction with low C:N ratio

403    suggesting a rather high biogeochemical stability (and low turnover rate) of SOC in these

404    mountain grassland soils. The contribution of this biogeochemical stable fraction (MAOM)

405 did not change across seasons contrary to the more labile C-pools (WEOC, C-POM) showing

406 high seasonal changes of their respective contributions to total SOC or/and their chemistry.

407 Surprisingly, the decrease in organic C content for the bulk soil induced by the climate

408 manipulation already observed by Puissant et al. (2015) was not explained by C-losses from

409 fast cycling C-pools (WEOC, freePOM or occPOM), but by significant C-losses in the

410 MAOM fraction after four years of climate change experiment. These findings challenged

411 our three main hypotheses and we discuss below their implications regarding the dynamics of

412 SOC pools in our climate change experiment.

413

414      4.1. Weak contributions of labile SOM fractions to total SOC

415      Contrary to our first hypothesis, the POM-C fractions did not represent a large

416 proportion of total SOC in these mountain grassland soils. This result contrasts with those

417 from other studies on mountain soils which found that a large proportion of total SOC

418 consisted of labile POM fractions (Budge et al., 2011; Leifeld et al., 2009; Martinsen et al.,

419 2011; Saenger et al., 2015). Indeed, Martinsen et al. (2011) reported that the POM fraction

420 contained more than 60% of total SOC in the first 5 cm of a low-alpine (1050-1320 m a.s.l.)

421 grasslands soil (with or without sheep grazing) from southern Norway. Similar results were

422 reported by Leifeld et al (2009) in the uppermost layers of high altitude (2200 m a.s.l.)

423 permanent mountain grasslands with more than 80% of total SOC contained in the POM

424 fraction. Saenger et al (2015) recently reported that 38 % of total SOC was found in the POM

425 fraction in a French mesic grassland on limestone at 1567 m a.s.l. grazed by sheep. By

426 combining several studies of Swiss mountain grasslands ranging in altitude from 1210 to

427 1410 m a.s.l. (i.e. similar to the studied grasslands in the Jura mountains), Leifeld et al (2009)

428 reported that the proportion of C-POM in the first 10 cm of soils ranged from ca. 24 to 27 %.

429    These latter results are closer to our findings. Leifeld and Kögel-Knabner (2005) and Meyer et

430    al (2012b) have shown that the land use and the management type could impact the relative

431    proportion of SOM fractions. Here the pasture management (grazing by cattle and then mown

432    during the four years experiment) could partly explain the low proportion of POM-C in these

433    mountain subalpine soils, with large parts of the herbaceous biomass taken up by grazing.

434    Such effect of grazing on POM-C has already been reported (Martinsen et al, 2011, but see

435    Leifeld and Fuhrer, 2009; Meyer et al 2012b). Overall, our results and those from the

436    literature suggest that altitude and grazing may interact to control the POM-C content of

437    mountain subalpine topsoils. The WEOC fraction yielded the smallest contribution to SOC

438    (0.09 to 0.34 %) and this result was consistent to other studies using similar methodologies

439    (see e.g. Rees and Parker, 2005). In this study, SOM consisted mostly of MAOM which has

440    achieved a higher degree of microbial transformation as attested by its low C:N ratio as

441    compared to the freePOM and occPOM fractions. A decrease in C:N ratio from labile (POM)

442    to the MAOM fraction is generally found (Budge et al, 2011;Golchin et al, 1994 ; Baisden et

443    al, 2002). High amounts of MAOM-C with low C:N ratio suggest a rather high

444    biogeochemical stability (and low turnover rate) of SOC in these mountain grassland soils,

445    which contrasts with current knowledge presenting mountain soils as huge reservoirs of labile

446    C (Sjögersten et al., 2011; Leifeld et al., 2009; Saenger et al., 2015).

447

448        4.2. Weak impact of the climate change experiment on the labile SOM fractions and

449            their seasonal dynamics

450    4.2.1. Seasonal dynamics of the labile SOM fractions

451        As we hypothesized, the strong seasonal climatic changes characterized by a winter

452    period with a continuous snow cover at all sites, and a warm and relatively dry summer

453 affected the dynamics of the most labile SOM fractions. Only the occPOM fraction

454 contribution was not affected by the seasonal change.

455       The WEOC fraction considered as the most available substrate for microorganisms

456 was very sensitive to seasonal climatic variations. Both, WEOC abundance and chemistry

457 were strongly impacted by seasonal changes. We observed globally higher WEOC contents in

458 the summer season combined with a more recalcitrant WEOC chemistry (higher UV

459 absorbance at 280nm and higher proportion of aromatic C, fulvic and humic C compared to

460 proteic C). Such temporal dynamics of WEOC content has been previously observed, with

461 higher WEOC content (Yano et al., 2000; Kaiser et al., 2001) and higher humic chemistry in

462 the summer season (Kaiser et al 2001, Qualls and Haines, 1992). However studies specifically

463 characterising the seasonal pattern of WEOC chemistry are few, and  contradictory patterns

464 have also  been reported (see the review by (Embacher et al., 2007). Our study identifies that

465 this seasonal pattern of WEOC abundance were decoupled from soil microbial abundance and

466 activity (Puissant et al, 2015). This contrasts with several other studies showing positive

467 correlations (Marschner and Bredow, 2002; Marschner and Kalbitz, 2003; Rees and Parker,

468 2005) and could indicate that WEOC abundance may not be the limiting factor for microbial

469 growth which is more affected by summer drought in these subalpine grasslands.

470       The freePOM fraction also showed an important seasonal pattern with a higher

471 contribution to total SOC at the summer sampling date. This result could be linked to the

472 seasonal root dynamics which is a significant component of soil C inputs in grasslands

473 (Gregorich et al., 2006; Hitz et al., 2001). Contrary to the freePOM fractions, the relative

474 contribution of the occPOM fraction to total SOC did not show any seasonal trend,

475 underlining its different biogeochemical reactivity compared to the freePOM fraction, as

476 previously reported by Meyer et al. (2011). Interestingly, the organic chemistry of the

477 freePOM and the occPOM fractions (mid-infrared spectroscopic proxies) showed a seasonal

478  pattern, as well as chemical differences between both POM-C fractions. Our results suggest

479  that fresh OM inputs (low Alkyl-C/Alkyl-O ratio) are incorporated into the freePOM fraction

480  during summer. In winter, elements of the freePOM are incorporated into the occPOM

481  fraction (lower Alkyl-C/Alkyl-O ratio in winter compared with summer for the occPOM

482  fraction). The seasonal difference in the chemistry of the occPOM fraction underlines the

483  ontogeny of plant detritus during the aggregation process on a seasonal timescale (Cécillon et

484  al., 2010; Moore et al., 2004).

485      4.2.2. Evolution of the labile SOM fractions induced by the climate manipulation

486      Contrary to our second hypothesis, the climate change manipulation did not strongly

487  affect the respective contributions of labile SOC pools (WEOC, freePOM and occPOM) to

488  total SOC. The only labile SOM fraction impacted by the climate manipulation experiment

489  was the freePOM fraction. A minor increase in the relative contribution of freePOM-C to total

490  SOC was observed at the lowest site (570m, only significant at the summer sampling date).

491  Here, the freePOM fraction could accumulate under climate manipulated conditions because

492  of the decreased microbial activity (enzyme pools and soil respiration) observed on the same

493  plots, probably due to the effect of water limitation at lower transplantation sites (Mills et al.,

494  2014; Puissant et al, 2015; Gavazov et al, 2014a). As a consequence, the effect of the climate

495  manipulation on the contribution of the labile SOM fractions (small increased contribution of

496  freePOM-C to total SOC) could not explain the overall losses in bulk SOC observed after four

497  years of experiment. Interestingly, the chemical differences between the freePOM and the

498  occPOM fractions were modulated by the climate manipulation (Supplementary Table 1),

499  suggesting a climate change induced modification of soil macro-aggregates dynamics. Other

500  studies have shown that physical occlusion of OM within soil aggregates could be affected by

501  the climate conditions like freeze-thaw cycles (Sollins et al., 1996). The modification of soil

502    macro-aggregates dynamics under climate change could have potentially important

503    repercussions on the long term soil C dynamics.

504

505            4.3. Large C-losses from the MAOM fraction under the climate change

506                 manipulation

507    Contrary to our third hypothesis, significant C-losses from the MAOM fraction, the most

508    biogeochemically stable fraction, were observed after four years of climate change

509    manipulation. These C-losses coincided with the decrease in organic C content for the bulk

510    soil already observed in the same experiment (Puissant et al, 2015) in the topsoil, which was

511    even more pronounced at greater soil depth (10-15 cm and 15-30 cm; Fig.S1). This climate-

512    induced modification on MAOM-C content was also linked to an important textural change,

513    with a decrease in clay content at the lower transplantation sites.

514    In order to understand this climate-induced modification of SOC content, it is necessary to

515    frame the observed SOM dynamics in an ecosystem context, considering both soil C inputs

516    and outputs. Previous investigations of the same soil transplantation experiment revealed the

517    following effects of the climate manipulation on SOM dynamics: (i) a significant decrease in

518    SOM outputs through respiration (Mills et al., 2014), which was confirmed by a decrease in

519    soil enzyme pools and soil microbial biomass (Puissant et al, 2015); (ii) an increase in SOM

520    outputs through dissolved organic matter leaching (+ 9.9 mg C $L^{-1}$ at the lowest site and + 4.2

521    mg C $L^{-1}$ at the intermediate site relative to high elevation controls; Gavazov, 2013); (iii)

522    irregular changes of aboveground SOM inputs (aboveground biomass production) depending

523    on the year (Table 3); (iv) a strong decrease in belowground SOM inputs through a huge

524    decrease of root biomass (Table 3).

525     In combination with these results, our present study illustrates that climate-manipulation

526    caused C losses through a reduction in belowground plant C inputs together with enhanced

527    leaching processes. The intense C-leaching may itself be linked to several observed changes

528    of soil parameters under the climate manipulation. Indeed, decreased root biomass and

529    microbial biomass and activity could have resulted in changes of soil structure and its

530    resistance to climate events such as precipitation events. Root biomass has been shown to be

531    an important factor controlling soil erosion and leaching (Gyssel et al, 2005). Moreover, the

532    microbial products of decomposition, reduced under the climate manipulation, are the main

533    precursors of stable SOM by promoting SOM formation, aggregation and association to

534    mineral matrix (Cotrufo et al, 2013, Malik et al, 2016). The observed modification of soil

535    macro-aggregates dynamics under climate change further coincide with this hypothesis. In

536    fact, such changes in soil structure could have increased clay dispersion which has then led to

537    a vertical migration of clay colloids down the mesocosms.

538     The important MAOM-C losses following the climate change manipulation may thus be

539    explained by washing out of clay-SOM associations leading to the strong difference in soil

540    texture and the increase in C-leaching observed (Gavazov, 2013). Losses of organic C through

541    leaching has been shown to be an important factor within the C cycle of grasslands (5 to 98 %

542    of C net ecosystem exchange; Kindler et al., 2011). However in our study, losses of clays and

543    associated MAOM-C following the climate change manipulation may have been artificially

544    enhanced by the use of mesocosms of 30 cm depth. In real grassland ecosystems, migration of

545    clay colloids and C-leaching could be stabilized at a greater soil depth, but it is likely that

546    some of the destabilized clay-SOM associations would be lost.

547

548    **Conclusion**

549

550     Our study shows that most of the SOC content of these subalpine grazed grassland soils

551     was contained in the stable MAOM fraction. As expected, the most labile C pools (WEOC

552     and freePOM) showed a high intra-annual dynamics (amounts and chemistry) mediated via

553     the seasonal changes of fresh plant debris inputs and confirming their high contribution to the

554     microbial loop. The amount of OM occluded into soil macro-aggregates was constant between

555     seasons and sites, whereas its chemistry changed. Such results confirmed the relatively short

556     turnover time of soil macro-aggregates and suggest an effect of the climate change

557     manipulation on soil macro-aggregate dynamics. Four years of reduced precipitation and

558     higher mean annual temperature led to a decrease of total SOC concentration due to a

559     decrease of SOC content in the MAOM fraction. The combined effects of the reduction in

560     plants inputs, roots biomass, microbial biomass and activity could have led to change the soil

561     structure and its resistance to climate events. The decrease in clay content and the increase in

562     DOC leaching under climate change manipulation support the hypothesis of C-losses form

563     MAOM fraction through clay-SOM washing out and DOC leaching in this subalpine

564     grassland. We ask for more studies to understand possible environmental cascade reactions

565     impacting the soil carbon cycle under a warming world. Our results highlight the importance

566     of (i) quantifying the seasonal dynamics of labile SOM pools and (ii) considering SOM

567     outputs linked with processes such as erosion or C-leaching so as to understand the fate of soil

568     carbon change under climate change.

569

23

584

585

586

24

## References

588   Baisden, W.T., Amundson, R., Cook, A.C., Brenner, D.L., 2002. Turnover and storage of C
589       and N in five density fractions from California annual grassland surface soils. Global
590       Biogeochemical Cycles 16, 64–1–64–16. doi:10.1029/2001GB001822

591   Baldock, J. A., Oades, J. M., Nelson, P. N., Skene, T. M., Golchin, A., and Clarke, P.:
592       Assessing the extent of decomposition of natural organic materials using solid-state 13C
593       NMR spectroscopy, Aust. J. Soil Res., 35, 1061–1083, 2007.

594   Beleites, C. and Sergo, V., 2012. HyperSpec: a package to handle hyperspectral data sets in R.
595       R package v. 0.98-20110927. http://hyperspec.r-forge.r-project.org

596   Bloemberg, T.G., Gerretzen, J., Wouters, H.J.P., Gloerich, J., van Dael, M., Wessels,
597       H.J.C.T., van den Heuvel, L.P., Eilers, P.H.C., Buydens, L.M.C. and Wehrens, R., 2010.
598       Improved parametric time warping for proteomics. Chemometrics and Intelligent
599       Laboratory Systems 104 (1), 65–74. doi:10.1016/j.chemolab.2010.04.008

600   Boddy, E., Hill, P.W., Farrar, J., Jones, D.L., 2007. Fast turnover of low molecular weight
601       components of the dissolved organic carbon pool of temperate grassland field soils. Soil
602       Biology and Biochemistry 39, 827–835.

603   Budge, K., Leifeld, J., Hiltbrunner, E., Fuhrer, J., 2011. Alpine grassland soils contain large
604       proportion of labile carbon but indicate long turnover times. Biogeosciences 8, 1911–
605       1923. doi:10.5194/bg-8-1911-2011

606   Burns, R.G., DeForest, J.L., Marxsen, J., Sinsabaugh, R.L., Stromberger, M.E., Wallenstein,
607       M.D., Weintraub, M.N., Zoppini, A., 2013. Soil enzymes in a changing environment:
608       Current knowledge and future directions. Soil Biology and Biochemistry 58, 216–234.
609       doi:10.1016/j.soilbio.2012.11.009

610   C2SM, 2011. Swiss Climate Change Scenarios CH2011. doi:10.3929/ethz-a-006720559

611   Carvalhais, N., Forkel, M., Khomik, M., Bellarby, J., Jung, M., Migliavacca, M., Mingquan,
612       M., Saatchi, S., Santoro, M., Thurner, M., Weber, U., Ahrens, B., Beer, C., Cescatti, A.,

613      Randerson, J.T., Reichstein, M., 2014. Climate in Terrestrial Ecosystems. Nature 514,

614      213–217. doi:10.1038/nature13731

615      Cécillon, L., Certini, G., Lange, H., Forte, C., Strand, L.T., 2012. Spectral fingerprinting of

616      soil organic matter composition. Organic Geochemistry 46, 127–136.

617      doi:10.1016/j.orggeochem.2012.02.006

618      Cécillon, L., Mello, N. a., Danieli, S., Brun, J.-J., 2010. Soil macroaggregate dynamics in a

619      mountain spatial climate gradient. Biogeochemistry 97, 31–43. doi:10.1007/s10533-009-

620      9341-9

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

622      Steinweg, J.M., 2008. Sensitivity of organic matter decomposition to warming varies

623      with its quality. Global Change Biology 14, 868–877. doi:10.1111/j.1365-

624      2486.2008.01541.x

625      Conant, R.T., Ryan, M.G., Ågren, G.I., Birge, H.E., Davidson, E.A., Eliasson, P.E., Evans,

626      S.E., Frey, S.D., Giardina, C.P., Hopkins, F.M., Hyvönen, R., Kirschbaum, M.U.F.,

627      Lavallee, J.M., Leifeld, J., Parton, W.J., Megan Steinweg, J., Wallenstein, M.D., Martin

628      Wetterstedt, J.Å., Bradford, M.A., 2011. Temperature and soil organic matter

629      decomposition rates - synthesis of current knowledge and a way forward. Global Change

630      Biology 17, 3392–3404. doi:10.1111/j.1365-2486.2011.02496.x

631      Cotrufo, M. F., Wallenstein, M. D., Boot, C. M., Denef, K. and Paul, E. (2013), The

632      Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter

633      decomposition with soil organic matter stabilization: do labile plant inputs form stable

634      soil organic matter?. Global Change Biology, 19: 988–995. doi: 10.1111/gcb.12113

635      Davidson, E. a, Janssens, I. a, 2006. Temperature sensitivity of soil carbon decomposition and

636      feedbacks to climate change. Nature 440, 165–73. doi:10.1038/nature04514

637      Signal Developers, 2013. Signal: signal processing. URL http://r-forge. r-project.

638      org/projects/signal

639      Eglin, T., Ciais, P., Piao, S.L., Barre, P., Bellassen, V., Cadule, P., Chenu, C., Gasser, T.,

640      Koven, C., Reichstein, M., Smith, P., 2010. Historical and future perspectives of global

641        soil carbon response to climate and land-use changes. Tellus 62B, 700–718.

642        doi:10.1111/j.1600-0889.2010.00499.x

643    Embacher, A., Zsolnay, A., Gattinger, A., Munch, J.C., 2007. The dynamics of water

644        extractable organic matter (WEOM) in common arable topsoils: I. Quantity, quality and

645        function over a three year period. Geoderma 139, 11–22.

646        doi:10.1016/j.geoderma.2006.12.002

647    Erktan A, Cécillon L, Graf F, Roumet C, Legout C, Rey F, 2016. Increase in soil aggregate

648        stability along a Mediterranean successional gradient in severely eroded gully bed

649        ecosystems: combined effects of soil, root traits and plant community characteristics.

650        Plant and Soil 398, 121-137

651    Frei, C., Schöll, R., Fukutome, S., Schmidli, J., Vidale, P.L., 2006. Future change of

652        precipitation extremes in Europe: Intercomparison of scenarios from regional climate

653        models. Journal of Geophysical Research 111, D06105. doi:10.1029/2005JD005965

654    Gavazov, K., Mills, R., Spiegelberger, T., Lenglet, J., Buttler, A., 2014a. Biotic and Abiotic

655        Constraints on the Decomposition of Fagus sylvatica Leaf Litter Along an Altitudinal

656        Gradient in Contrasting Land-Use Types. Ecosystems 17, 1326–1337.

657        doi:10.1007/s10021-014-9798-9

658    Gavazov, K., Spiegelberger, T., Buttler, A., 2014b. Transplantation of subalpine wood-

659        pasture turfs along a natural climatic gradient reveals lower resistance of unwooded

660        pastures to climate change compared to wooded ones. Oecologia 174, 1425–35.

661        doi:10.1007/s00442-013-2839-9

662    Gavazov, K.S., 2013. Subalpine pasture-woodlands in a changing climate: The role of land

663        use type in ecosystem resistance to simulated climate change. Thesis EPFL 5989.

664        Lausanne, Swizerland. doi: 10.5075/epfl-thesis-5989

665    Gavazov, K., Peringer, A., Buttler, A., Gillet, F., Spiegelberger, T., 2013. Dynamics of Forage

666        Production in Pasture-woodlands of the Swiss Jura Mountains under Projected Climate

667        Change Scenarios. Ecology and Society 18, art38. doi:10.5751/ES-04974-180138

668     Gobiet, A., Kotlarski, S., Beniston, M., Heinrich, G., Rajczak, J., Stoffel, M., 2014. 21st

669          century climate change in the European Alps-A review. Science of the Total

670          Environment 493, 1138–1151. doi:10.1016/j.scitotenv.2013.07.050

671     Golchin, A., Oades, J. M., Skjemstad, J. O., and Clarke, P.: Study of free and occluded

672          particulate organic matter in soils by solid state 13CCP/MASNMRspectroscopy and

673          scanning electron mi- croscopy, Aust. J. Soil Res., 32, 285–309, 1994.Gregorich, E.G.,

674          Beare, M.H., McKim, U.F., Skjemstad, J.O., 2006. Chemical and Biological

675          Characteristics of Physically Uncomplexed Organic Matter. Soil Science Society of

676          America Journal 70, 975–985. doi:10.2136/sssaj2005.0116

677     Groffman PM, Driscoll CT, Fahey TJ et al (2001) Colder soils in a warmer world: a snow

678          manipulation study in a northern hardwood forest ecosystem. Biogeochemistry 56:135–

679          150

680     Guigue, J., Mathieu, O., Lévêque, J., Mounier, S., Laffont, R., Maron, P. a., Navarro, N.,

681          Chateau, C., Amiotte-Suchet, P., Lucas, Y., 2014. A comparison of extraction procedures

682          for water-extractable organic matter in soils. European Journal of Soil Science 65, 520–

683          530. doi:10.1111/ejss.12156

684     Gyssels G., Poesen J., Bochet E., Li Y., 2005. Impact of plant roots on the resistance of soils

685          to erosion by water: a review. Progress in Physical Geography 29: 189-217,

686          doi:10.1191/0309133305pp443ra

687     Helfrich, M., Ludwig, B., Buurman, P., Flessa, H., 2006. Effect of land use on the

688          composition of soil organic matter in density and aggregate fractions as revealed by

689          solid-state 13C NMR spectroscopy. Geoderma 136, 331–341.

690          doi:10.1016/j.geoderma.2006.03.048

691     Hitz, C., Egli, M., Fitze, P., 2001. Below-ground and above-ground production of

692          vegetational organic matter along a climosequence in alpine grasslands. Journal of Plant

693          Nutrition and Soil Science 164, 389–397.

694     IUSS Working Group WRB. 2007. World Reference Base for Soil Resources 2006, first

695          update 2007. World Soil Resources Reports No. 103. FAO, Rome., n.d.

696 John, B., Yamashita, T., Ludwig, B., Flessa, H., 2005. Storage of organic carbon in aggregate
697     and density fractions of silty soils under different types of land use, in: Geoderma. pp.
698     63–79.

699 Kaiser, K., Guggenberger, G., Haumaier, L., Zech, W., 2001. Seasonal variations in the
700     chemical composition of dissolved organic matter in organic forest floor layer leachates
701     of old-growth Scots pine (Pinus sylvestris L.) and European beech (Fagus sylvatica L.)
702     stands in northeastern Bavaria, Germany.Biogeochemistry 55 (2), 103-143

703 Kalbitz, K., Schmerwitz, J., Schwesig, D., Matzner, E., 2003. Biodegradation of soil-derived
704     dissolved organic matter as related to its properties. Geoderma 113, 273–291.
705     doi:10.1016/S0016-7061(02)00365-8

706 Kindler, R., Siemens, J., Kaiser, K., Walmsley, D.C., Bernhofer, C., Buchmann, N., Cellier,
707     P., Eugster, W., Gleixner, G., Grunwald, T., Heim, A., Ibrom, A., Jones, S.K., Jones, M.,
708     Klumpp, K., Kutsch, W., Larsen, K.S., Lehuger, S., Loubet, B., Mckenzie, R., Moors, E.,
709     Osborne, B., Pilegaard, K., Rebmann, C., Saunders, M., Schmidt, M.W.I., Schrumpf, M.,
710     Seyfferth, J., Skiba, U., Soussana, J.F., Sutton, M.A., Tefs, C., Vowinckel, B., Zeeman,
711     M.J., Kaupenjohann, M., 2011. Dissolved carbon leaching from soil is a crucial
712     component of the net ecosystem carbon balance. Global Change Biology 17, 1167–1185.

713 Kögel-Knabner, I., 2002. The macromolecular organic composition of plant and microbial
714     residues as inputs to soil organic matter. Soil Biology & Biochemistry 34, 139–162.

715 Kölbl, A., Kögel-Knabner, I., 2004. Content and composition of free and occluded particulate
716     organic matter in a differently textured arable Cambisol as revealed by solid-state 13C
717     NMR spectroscopy. Journal of Plant Nutrition and Soil Science 167, 45–53.
718     doi:10.1002/jpln.200321185

719 Kölbl, A., Leifeld, J., Kögel-Knabner, I., 2005. A comparison of two methods for the isolation
720     of free and occluded particulate organic matter. Journal of Plant Nutrition and Soil
721     Science 168, 660–667. doi:10.1002/jpln.200521805

722 Lehmann, J., Solomon, D., Kinyangi, J., Dathe, L., Wirick, S., Jacobsen, C., 2008. Spatial
723     complexity of soil organic matter forms at nanometre scales. Nature Geoscience 1, 238–
724     242. doi:10.1038/ngeo155

725  Leifeld, J., Fuhrer, J., 2009. Long-term management effects on soil organic matter in two
726      cold, high-elevation grasslands: clues from fractionation and radiocarbon dating.
727      European Journal of Soil Science 60, 230–239. doi:10.1111/j.1365-2389.2008.01111.x

728  Leifeld, J., Kögel-Knabner, I., 2005. Soil organic matter fractions as early indicators for
729      carbon stock changes under different land-use? Geoderma 124, 143–155.
730      doi:10.1016/j.geoderma.2004.04.009

731  Leifeld, J., Zimmermann, M., Fuhrer, J., Conen, F., 2009. Storage and turnover of carbon in
732      grassland soils along an elevation gradient in the Swiss Alps. Global Change Biology 15,
733      668–679. doi:10.1111/j.1365-2486.2008.01782.x

734  Malik, A., Roth, V.-N., Hébert, M., Tremblay, L., Dittmar, T., & Gleixner, G. (2016).Linking
735      molecular size, composition and carbon turnover of extractable soil microbial
736      compounds. Soil Biology and Biochemistry, 100, 66-73.
737      doi:10.1016/j.soilbio.2016.05.019.

738  Manzoni, S., Porporato, A., 2009. Soil carbon and nitrogen mineralization: Theory and
739      models across scales. Soil Biology and Biochemistry 41, 1355–1379.
740      doi:10.1016/j.soilbio.2009.02.031

741  Marschner, B., Bredow, A., 2002. Temperature effects on release and ecologically relevant
742      properties of dissolved organic carbon in sterilised and biologically active soil samples.
743      Soil Biology and Biochemistry 34, 459–466.

744  Marschner, B., Kalbitz, K., 2003. Controls of bioavailability and biodegradability of dissolved
745      organic matter in soils. Geoderma 113, 211–235. doi:10.1016/S0016-7061(02)00362-2

746  Martinsen, V., Mulder, J., Austrheim, G., Mysterud, a., 2011. Carbon storage in low-alpine
747      grassland soils: effects of different grazing intensities of sheep. European Journal of Soil
748      Science 62, 822–833. doi:10.1111/j.1365-2389.2011.01393.x

749  Meehl, G.A., Stocker, T.F., Collins, W.D., Friedlingstein, P., Gaye, A.T., Gregory, J.M.,
750      Kitoh, A., R. Knutti, J.M. Murphy, A. Noda, S.C.B. Raper, I.G. Watterson, A.J. Weaver
751      and Z.-C. ZhaoMeehl, G.A., T.F. Stocker, W.D. Collins, P. Friedlingstein, A.T. Gaye,
752      J.M. Gregory, A. Kitoh, R. Knutti, J.M. Murphy, A. Noda, S.C.B. Raper, I.G. Watterson,

753     A.J.W. and Z.-C.Z., 2007. The Physical Science Basis. Contribution of Working Group I
754     to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change
755     [Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor and
756     H.L. Miller (eds.)]. Cambridge U.

757     Meyer, S., Leifeld, J., Bahn, M., Fuhrer, J., 2011. Free and protected soil organic carbon
758     dynamics respond differently to abandonment of mountain grassland. Biogeosciences
759     Discussions 8, 9943–9976. doi:10.5194/bgd-8-9943-2011

760     Meyer, S., Leifeld, J., Bahn, M., Fuhrer, J., 2012a. Free and protected soil organic carbon
761     dynamics respond differently to abandonment of mountain grassland. Biogeosciences 9,
762     853–865. doi:10.5194/bg-9-853-2012

763     Meyer, S., Leifeld, J., Bahn, M., Fuhrer, J., 2012b. Land-use change in subalpine grassland
764     soils: Effect on particulate organic carbon fractions and aggregation. Journal of Plant
765     Nutrition and Soil Science 175, 401–409. doi:10.1002/jpln.201100220

766     Mills, R.T.E., Gavazov, K.S., Spiegelberger, T., Johnson, D., Buttler, A., 2014. Diminished
767     soil functions occur under simulated climate change in a sup-alpine pasture, but
768     heterotrophic temperature sensitivity indicates microbial resilience. The Science of the
769     total environment 473-474, 465–72. doi:10.1016/j.scitotenv.2013.12.071

770     Moore, J.C., Berlow, E.L., Coleman, D.C., Ruiter, P.C., Dong, Q., Hastings, A., Johnson,
771     N.C., McCann, K.S., Melville, K., Morin, P.J., Nadelhoffer, K., Rosemond, A.D., Post,
772     D.M., Sabo, J.L., Scow, K.M., Vanni, M.J., Wall, D.H., 2004. Detritus, trophic dynamics
773     and biodiversity. Ecology Letters 7, 584–600. doi:10.1111/j.1461-0248.2004.00606.x

774     Moyano, F.E., Vasilyeva, N., Bouckaert, L., Cook, F., Craine, J., Curiel Yuste, J., Don, a.,
775     Epron, D., Formanek, P., Franzluebbers, a., Ilstedt, U., Kätterer, T., Orchard, V.,
776     Reichstein, M., Rey, a., Ruamps, L., Subke, J. a., Thomsen, I.K., Chenu, C., 2012. The
777     moisture response of soil heterotrophic respiration: Interaction with soil properties.
778     Biogeosciences 9, 1173–1182. doi:10.5194/bg-9-1173-2012

779     NF ISO 16586, Soil quality., 2003. Determination of soil water content as a volume fraction
780     on the basis of known dry bulk density - Gravimetric method. AFNOR

781  NF ISO 11464, Soil quality., 2006. Pretreatment of samples for physico-chemical analysis.
782      AFNOR

783  NF X31-107, Soil quality, 2003. Determination of particle size distribution of the soil
784      particles - Method pipetted. AFNOR

785  NF ISO 10390, Soil quality, 2005. Determination of pH. AFNOR

786  NF ISO 10693, Soil quality., 1995. Determination of carbonate content. Volumetric method.
787      AFNOR

788  NF X31-130, Soil quality, 1999. Determination of cation exchange capacity (CEC) and the
789      extractable cations. AFNOR

790  NF ISO 10694, Soil quality., 1995. Determination of organic and total carbon after dry
791      combustion (elementary analysis). AFNOR

792  NF ISO 13878, Soil quality., 1995. Determination of total nitrogen content by dry combustion
793      ("elemental analysis"). AFNOR

794  Pengerud, A., Cécillon, L., Johnsen, L.K., Rasse, D.P., Strand, L.T., 2013. Permafrost
795      Distribution Drives Soil Organic Matter Stability in a Subarctic Palsa Peatland.
796      Ecosystems 16, 934–947. doi:10.1007/s10021-013-9652-5

797  Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., Core, T.R., 2014. nlme: Linear and Nonlinear
798      Mixed Effects Models. R package version 3.1-117, http://CRAN.R-
799      project.org/package=nlme.

800  Plante, A.F., Feng, Y., McGill, W.B., 2002. A modeling approach to quantifying soil
801      macroaggregate dynamics. Canadian Journal of Soil Science.

802  Poirier, N., Sohi, S., Gaunt, J., Mahieu, N., Randall, E., Powlson, D., Evershed, R., 2005. The
803      chemical composition of measurable soil organic matter pools. Organic Geochemistry
804      36, 1174–1189. doi:10.1016/j.orggeochem.2005.03.005

805  Puissant, J., Cécillon, L., Mills, R.T.E., Robroek, B.J.M., Gavazov, K., De Danieli, S.,
806      Spiegelberger, T., Buttler, A., Brun, J.-J., 2015. Seasonal influence of climate

807      manipulation on microbial community structure and function in mountain soils. Soil

808      Biology and Biochemistry 80, 296–305. doi:10.1016/j.soilbio.2014.10.013

809    Qualls, R.G., Haines, B.L., 1992. Biodegradability of Dissolved Organic Matter in Forest

810      Throughfall, Soil Solution, and Stream Water. Soil Science Society of America Journal.

811    Randerson, J.T., Masiello, C. a., Still, C.J., Rahn, T., Poorter, H., Field, C.B., 2006. Is carbon

812      within the global terrestrial biosphere becoming more oxidized? Implications for trends

813      in atmospheric O2. Global Change Biology 12, 260–271. doi:10.1111/j.1365-

814      2486.2006.01099.x

815    Robroek, B. J. M., Jassey, V. E. J., Kox, M. A. R., Berendsen, R. L., Mills, R. T. E., Cécillon,

816      L., Puissant, J., Meima-Franke, M., Bakker, P. A. H. M. et Bodelier, P. L. E. (2015),

817      Peatland vascular plant functional types affect methane dynamics by altering microbial

818      community structure. Journal of Ecology 103, 925–934. doi: 10.1111/1365-2745.12413

819    Rees, R.M., Parker, J., 2005. Filtration increases the correlation between water extractable

820      organic carbon and soil microbial activity. Soil Biology & Biochemistry 37, 2240–2248.

821      doi:10.1016/j.soilbio.2005.03.024

822    Saenger, A., Cécillon, L., Poulenard, J., Bureau, F., De Daniéli, S., Gonzalez, J.-M., Brun, J.-

823      J., 2015. Surveying the carbon pools of mountain soils: A comparison of physical

824      fractionation and Rock-Eval pyrolysis. Geoderma 241-242, 279–288.

825      doi:10.1016/j.geoderma.2014.12.001

826    Saenger, A., Cécillon, L., Sebag, D., Brun, J.-J., 2013. Soil organic carbon quantity, chemistry

827      and thermal stability in a mountainous landscape: A Rock–Eval pyrolysis survey.

828      Organic Geochemistry 54, 101−114. doi:10.1016/j.orggeochem.2012.10.008

829    Schmidt, M. W. I., Rumpel, C. and Kögel-Knabner, I. (1999), Evaluation of an ultrasonic

830      dispersion procedure to isolate primary organomineral complexes from soils. European

831      Journal of Soil Science, 50: 87–94. doi: 10.1046/j.1365-2389.1999.00211.x

832    Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I. a.,

833      Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D. a. C., Nannipieri, P., Rasse,

834       D.P., Weiner, S., Trumbore, S.E., 2011. Persistence of soil organic matter as an

835       ecosystem property. Nature 478, 49–56. doi:10.1038/nature10386

836   Siano, D.B., Metzler, D.E., 1969. Band shapes of the electronic spectra of complex molecules.

837       J. Chem. Phys. 51, 1856–1861.

838   Signal developers (2013). signal: Signal processing. URL: http://r-forge.r-

839       project.org/projects/signal/.

840   Six, J., Bossuyt, H., Degryze, S., Denef, K., 2004. A history of research on the link between

841       (micro)aggregates, soil biota, and soil organic matter dynamics. Soil and Tillage

842       Research 79, 7–31. doi:10.1016/j.still.2004.03.008

843   Six, J., Conant, R.T., Paul, E.A., Paustian, K., 2002. Stabilization mechanisms of soil organic

844       matter : Implications for C-saturation of soils. Plant and Soil 241, 155–176.

845   Sjögersten, S., Alewell, C., Cécillon, L., Hagedorn, F., Jandl, R., Leifeld, J., Martinsen, V.,

846       Schindlbacher, A., Sebastian, M.T., Van Miegroet, H., 2011. Mountain Soils in a

847       Changing Climate - Vulnerability of Carbon Stocks and Ecosystem Feedbacks, in: Soil

848       Carbon in Sensitive European Ecosystems: From Science to Land Management. John

849       Wiley and Sons, pp. 118–148. doi:10.1002/9781119970255.ch6

850   Sohi, S.P., Mahieu, N., Arah, J.R.M., Powlson, D.S., Madari, B., Gaunt, J.L., 2001. A

851       Procedure for Isolating Soil Organic Matter Fractions Suitable for Modeling. Soil

852       Science Society of America Journal 65, 1121–1128.

853   Sollins, P., Swanston, C., Kramer, M., 2007. Stabilization and destabilization of soil organic

854       matter—a new focus. Biogeochemistry 85, 1–7. doi:10.1007/s10533-007-9099-x

855   Sollins, P., Homann, P. & Caldwell, B. A. Stabilization and destabilization of soil organic

856       matter: mechanisms and controls. Geoderma 74, 65–-105 (1996).

857   Stockmann, U., Adams, M. a., Crawford, J.W., Field, D.J., Henakaarchchi, N., Jenkins, M.,

858       Minasny, B., McBratney, A.B., Courcelles, V.D.R. De, Singh, K., Wheeler, I., Abbott,

859       L., Angers, D. a., Baldock, J., Bird, M., Brookes, P.C., Chenu, C., Jastrow, J.D., Lal, R.,

860       Lehmann, J., O'Donnell, A.G., Parton, W.J., Whitehead, D., Zimmermann, M., 2013.

861 The knowns, known unknowns and unknowns of sequestration of soil organic carbon.

862 Agriculture, Ecosystems & Environment 164, 80–99. doi:10.1016/j.agee.2012.10.001

863 Toberman, H., Evans, C.D., Freeman, C., Fenner, N., White, M., Emmett, B. a., Artz, R.R.E.,

864 2008. Summer drought effects upon soil and litter extracellular phenol oxidase activity

865 and soluble carbon release in an upland Calluna heathland. Soil Biology & Biochemistry

866 40, 1519–1532. doi:10.1016/j.soilbio.2008.01.004

867 Torn, M.S., Swanston, C.W., Castanha, C., Trumbore, S.E., 2009. 6. Storage and turnover of

868 organic matter in soil, in: Biophysico-Chemical Processes Involving Natural Nonliving

869 Organic Matter in Environmental Systems. doi:10.1002/9780470494950.ch6

870 Von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner,

871 E., Marschner, B., 2007. SOM fractionation methods: Relevance to functional pools and

872 to stabilization mechanisms. Soil Biology & Biochemistry 39, 2183–2207.

873 doi:10.1016/j.soilbio.2007.03.007

874 Von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G.,

875 Marschner, B., Flessa, H., 2006. Stabilization of organic matter in temperate soils:

876 mechanisms and their relevance under different soil conditions - a review. European

877 Journal of Soil Science 57, 426–445. doi:10.1111/j.1365-2389.2006.00809.x

878 Yano, Y., McDowell, W.H., Aber, J.D., 2000. Biodegradable dissolved organic carbon in

879 forest soil solution and effects of chronic nitrogen deposition. Soil Biology &

880 Biochemistry 32, 1743–1751. doi:DOI: 10.1016/S0038-0717(00)00092-4

881 Zsolnay, Á., 2003. Dissolved organic matter: Artefacts, definitions, and functions. Geoderma

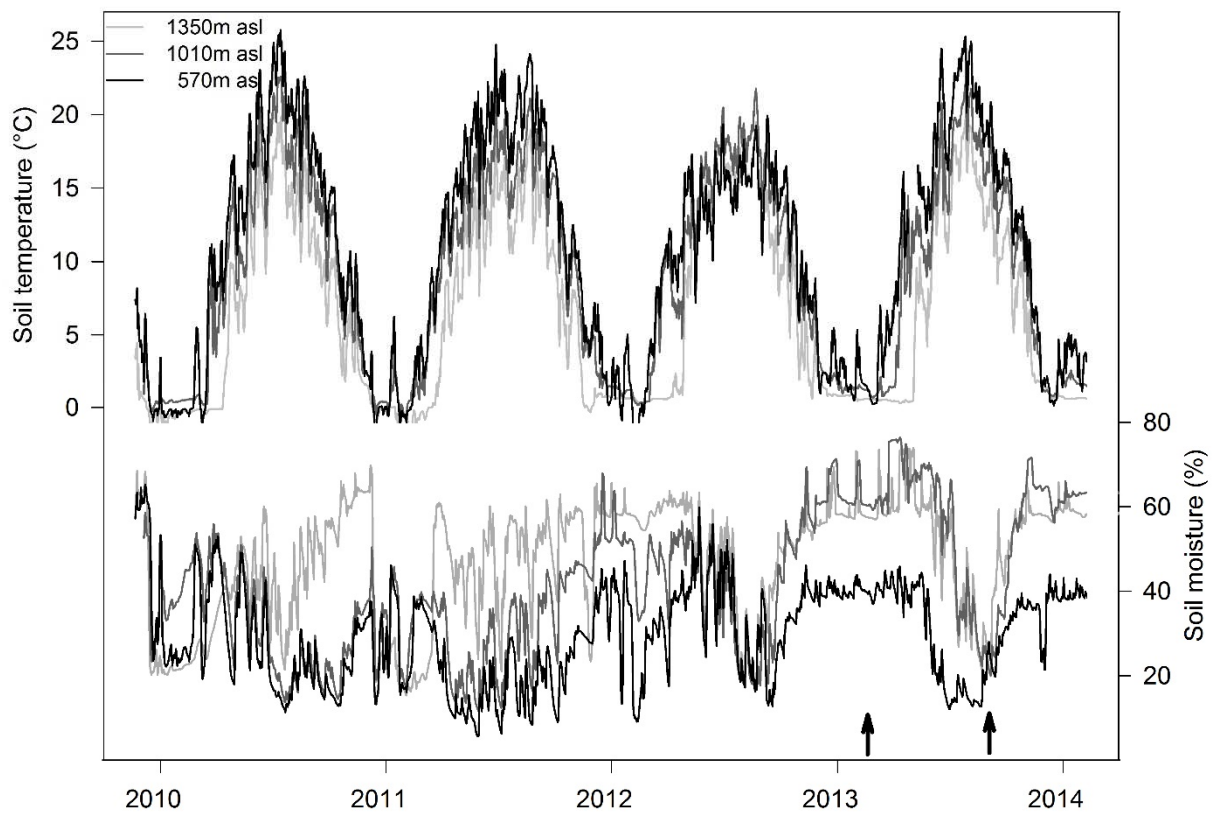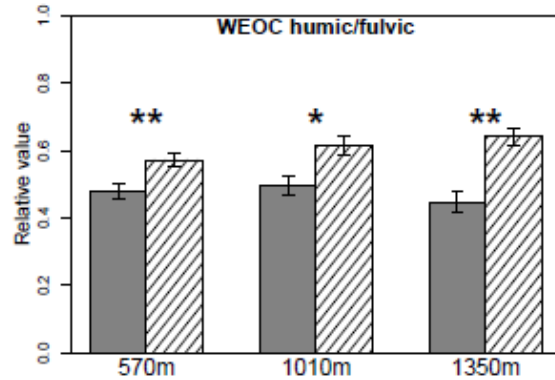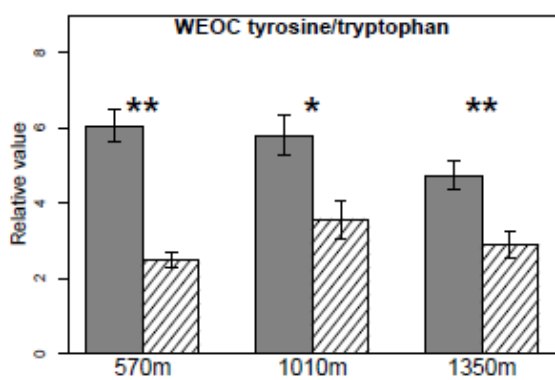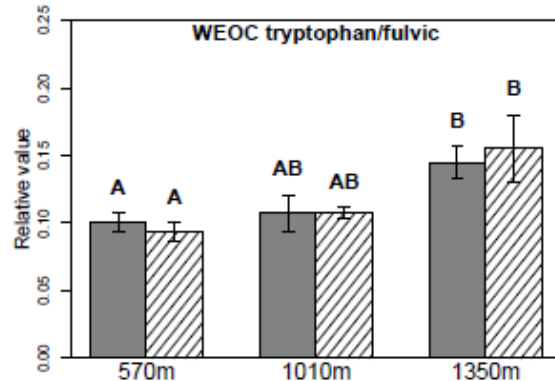882 113, 187–209. doi:10.1016/S0016-7061(02)00361-0

883

884
885

**Fig.1. Daily mean soil temperature and moisture of soil mesocosms at each site from the beginning of the experiment (2009) to the year 2014.** Control site (1350 m a.s.l.) in light grey line intermediate site (1010 m a.s.l.) in grey line; lowest site (570 m a.s.l.) in dark line. The two black arrows indicate the winter and summer dates of soil sampling in 2013.
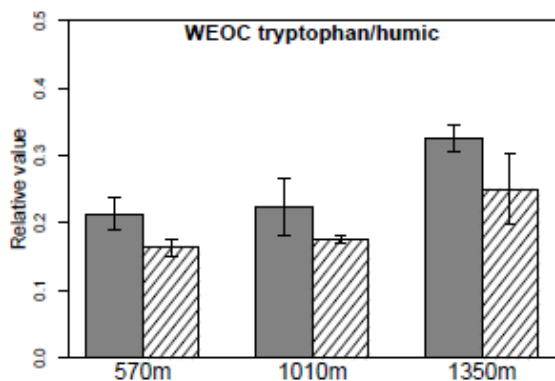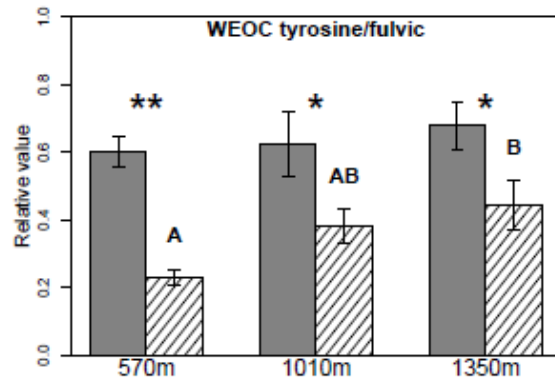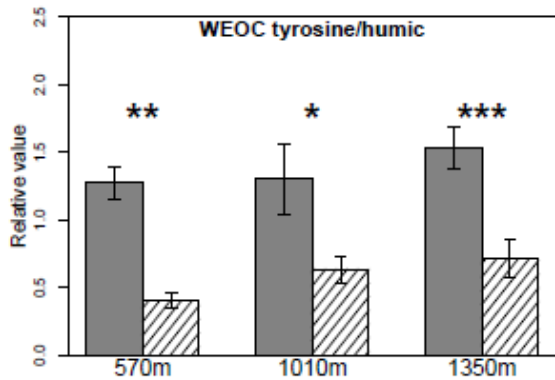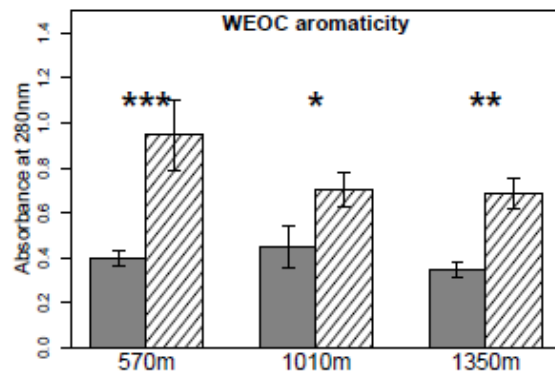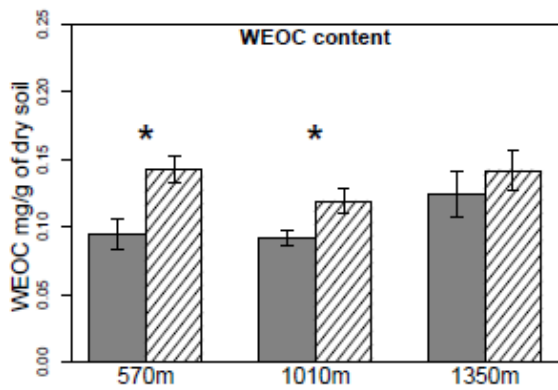
**Fig.2. Water extractable organic carbon (WEOC) abundance and chemistry (UV absorbance and fluorescence)**. Protein-like fluorophores for tyrosine, tryptophane, fulvic and humic compounds were simulated (see methods). Control site (1350 m a.s.l.); intermediate recipient site (1010 m a.s.l.); lowest recipient site (570 m a.s.l.). Asterisk symbols indicate significant differences between winter and summer season at each site (* for $p < 0.05$, ** for $p < 0.01$; *** for $p < 0.001$). Uppercase letters indicate significant differences between transplantation sites (climate manipulation) at each sampling date (season) ($p < 0.05$, climate manipulation effect)
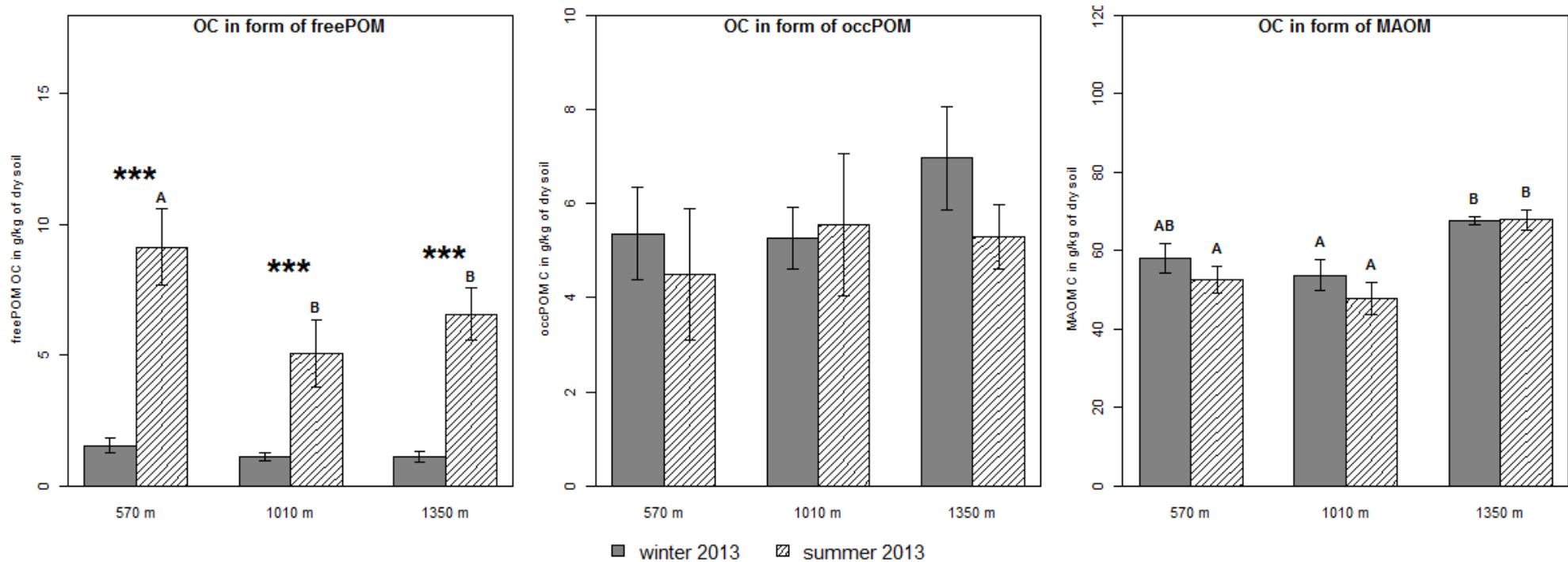
**Fig.3. Contribution of freePOM, occPOM and MAOM SOC fractions to bulk soil (gC / kg dry bulk soil)**. Control site (1350 m a.s.l.); intermediate site (1010 m a.s.l.); lowest site (570 m a.s.l.). Post hoc HSD tests are represented with different bold letters to indicate significant differences (p<0.05). Uppercase letters indicate significant differences between transplantation sites for each sampling date (winter or summer). Asterisk symbols indicate significant differences between winter and summer season at each site (* for p<0.05, ** for p<0.01; *** for p<0.001)

| Sampling dates | | Winter | | | Summer | | |
|---|---|---|---|---|---|---|---|
| | | (February 20th, 2013) | | | (September 2nd, 2013) | | |
| Site | m a.s.l. | 570 | 1010 | 1350 | 570 | 1010 | 1350 |
| Soil moisture | % | 43 ± 2*A* | 44 ± 2*A* | 50 ± 2*A* | 21 ± 1*B a* | 26 ± 2*B a* | 33 ± 1*B b* |
| Soil temperature | 0C | 1,2 ± 0,4*A* | 1,2 ± 0,2*A* | 0,6 ± 0,2*A* | 18,4 ± 0,4*B a* | 16,1 ± 0,1*B b* | 13,2 ± 0,2*B c* |
| pH | - | 5,7 ± 0,2*A* | 5,9 ± 0,2 | 5,7 ± 0 | 5,4 ± 0,2*B* | 5,7 ± 0,2 | 5,6 ± 0,1 |
| Calcareous | g.kg-1 | 2,6 ± 0,2 | 2,2 ± 0,2 | 2,2 ± 0,4 | 3,6 ± 1,1 | 2,4 ± 0,2 | 1,8 ± 0,2 |
| CEC | cmol.kg-1 | 33,9 ± 3,7A | 32,2 ± 4,5 | 38,4 ± 1,5 | 26,2 ± 2,3B | 29 ± 4 | 36,6 ± 1,7 |
| SOC | g.kg-1 | 66,5 ± 4,3ab | 60,7 ± 4,6b | 77,5 ± 2,1a | 69,8 ± 6,7a | 57,2 ± 5,3a | 81,1 ± 4,1b |
| Soil total nitrogen | g.kg-1 | 6,1 ± 0,4ab | 5,7 ± 0,5a | 7,4 ± 0,2b | 5,8 ± 0,4a | 5,2 ± 0,5a | 7,5 ± 0,4b |
| C/N | | 10,8 ± 0,2 | 10,7 ± 0,2 | 10,5 ± 0,1 | 12 ± 0,8 | 10,9 ± 0,2 | 10,8 ± 0,1 |
| Clay | g.kg-1 | | - | | 445 ± 40*ab* | 402 ± 22*a* | 526 ± 9*b* |
| Silt | g.kg-1 | | - | | 280 ± 16 | 283 ± 14 | 291 ± 6 |
| Sand | g.kg-1 | | - | | 275 ± 56 | 315 ± 30 | 182 ± 10 |

**Table 1: Basic soil characteristics for winter and summer sampling dates in the experimental sites.** The highest elevated site (1350 m a.s.l.) corresponds to the control site, and the two sites at 1010 and 570 m a.s.l. to the transplanted recipient sites. Values represent the mean (n=5) with the associated standard error (SE). Post hoc HSD tests are only represented with different bold letters to indicate significant differences (p<0.05). Uppercase letters indicate significant differences between sampling dates (winter/summer) at each site. Lowercase letters indicate significant differences between sites (manipulation effect) at each sampling date.

| Sampling dates | Winter (February 20th 2013) | | | Summer (September 2nd 2013) | | |
|---|---|---|---|---|---|---|
| Site (meters) | 570 | 1010 | 1350 | 570 | 1010 | 1350 |
| **OC content (g.kg of dry fraction)** | | | | | | |
| free POM | **267 ± 14a** | 285 ± 15 | **309 ± 14A** | 243 ± 11 | 256 ± 6 | **256 ± 4B** |
| occluded POM | **329 ± 6b A** | 294 ± 8A | 307 ± 8A | 258 ± 3B | 247 ± 18B | 231 ± 11B |
| **C/N** | | | | | | |
| free POM | 19 ± 1a | 19 ± 2a | 19 ± 1a | 19 ± 1a | **20 ± 1a** | 19 ± 1a |
| occluded POM | 18 ± 0a | 18 ± 1a | 18 ± 0a | 17 ± 0a | **18 ± 1b** | 18 ± 0a |
| MAOM | **10 ± 0c** | **10 ± 0c** | **10 ± 0c** | **10 ± 0c** | **10 ± 0c** | **10 ± 0c** |
| **HI index (humification)** | | | | | | |
| free POM | 6.1 ± 0.2 | 6.5 ± 1 | 7 ± 0.6 | **5.4 ± 0.3a** | **6.2 ± 0.5a** | 5.7 ± 0.2 |
| occluded POM | 6 ± 0.3 | **6.1 ± 0.6A** | 5.6 ± 0.6 | **8.1 ± 0.9b** | **8.7 ± 0.8b B** | 7 ± 0.5 |
| **C=O/SOC (oxidation)** | | | | | | |
| free POM | **9.8 ± 0.9A α** | **12.8 ± 2.9a αβ** | **18.8 ± 2.7a A β** | **4.7 ± 0.9B** | 7.6 ± 1.6 | **7.4 ± 0.6B** |
| occluded POM | 7.3 ± 0.9 | **6.1 ± 0.7b** | **5.9 ± 1.2b** | 6.8 ± 1 | 7.4 ± 1.1 | 5.9 ± 0.7 |
| **C=C/SOC (aromaticity)** | | | | | | |
| free POM | 3.3 ± 0.4 | 2.7 ± 0.6 | **1.7 ± 0.6A** | 3.3 ± 0.2 | 3.5 ± 0.5 | **3.9 ± 0.2B** |
| occluded POM | 3.1 ± 0.3 | 2.9 ± 0.3 | **3.9 ± 0.3b** | 3 ± 0.4 | 3.2 ± 0.7 | 3.6 ± 0.2 |
| **MI Index (maturation)** | | | | | | |
| free POM | 2.5 ± 0.4 | **2.1 ± 0.6a** | **1 ± 0.5aA** | 4.3 ± 0.7 | 3.3 ± 0.6 | **3.5 ± 0.2B** |
| occluded POM | 3 ± 0.4 | **3.3 ± 0.5b** | **4.2 ± 0.5b** | 3.2 ± 0.6 | 3.1 ± 0.8 | 3.9 ± 0.5 |
| **PI index (polysaccharide content)** | | | | | | |
| free POM | 8.5 ± 0.4 | 7.6 ± 0.7 | 9.8 ± 0.8 | 8 ± 0.2 | 9.5 ± 0.9 | 9.5 ± 0.3 |
| occluded POM | 8.2 ± 0.3 | 8 ± 0.7 | **8.1 ± 0.6A** | 8.5 ± 0.4 | 9.5 ± 0.6 | **10 ± 0.6B** |
| **CI Index (condensation)** | | | | | | |
| free POM | 6.3 ± 1.5 | 10.9 ± 4.6 | **15.6 ± 3.1a A** | **3.3 ± 0.7a** | **6 ± 0.9a** | **4.1 ± 0.5a B** |
| occluded POM | **2.5 ± 0.8A** | **5 ± 1.4A** | **3.1 ± 0.8b A** | **12 ± 1.6b B** | **18.5 ± 5.9b B** | **13.6 ± 2.8b B** |
| **Alkyl-C/Alkyl-O (microbial transformation)** | | | | | | |
| free POM | 7.2± 0.7 | **7.6 ± 0.1A** | 7.1± 0.2 | **6.7 ± 0.4a** | **6.6 ± 0.4a B** | 6 ± 0.2 |
| occluded POM | **7.3 ± 0.3A** | 7.6 ± 0.1 | 6.9 ± 0.4 | **9.6 ± 1.2b B** | **9.2 ± 1.2b** | 7 ± 0.3 |

**Table 2: Chemical characteristics of freePOM, occPOM and MAOM fractions of SOC.** Organic content of the fractions expressed in g Kg$^{-1}$ of SOC, CN ratios and proxies of POM bulk chemistry calculated from MIR analysis are given. Mean values (n=5) with the associated standard error (SE) are given. Post hoc HSD tests are represented with different bold letters to indicate significant differences (p<0.05). Uppercase letters indicate significant differences between sampling dates (winter/summer) at each site and for each fraction. Lowercase letters indicate significant differences between fractions at each sampling date and site. Greek letters indicate significant differences between transplantation sites

| Sampling year | Biomass | Unity | Site | | | Comparison to control site (%) | | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | 1350 m | 1010 m | 570 m | 1010 m | 570 m | |
| **2010** | | | 416 | 375 | 295 | -10 | -29 | Gavazov et al., 2013 |
| **2011** | Aboveground | | 250 | 240 | 95 | -4 | -62 | Gavazov et al., 2014b |
| **2012** | | g.m² | 233 | 368 | 414 | 58 | 78 | Gavazov 2013 |
| **2014** | | | 426 | 561 | 505 | 32 | 19 | *unpublished* |
| **2014** | Belowground | | 1890 | 967 | 751 | -49 | -60 | *unpublished* |

**Table 3: Mean aboveground and belowground biomass (g.m²) per year at each transplanted site.**
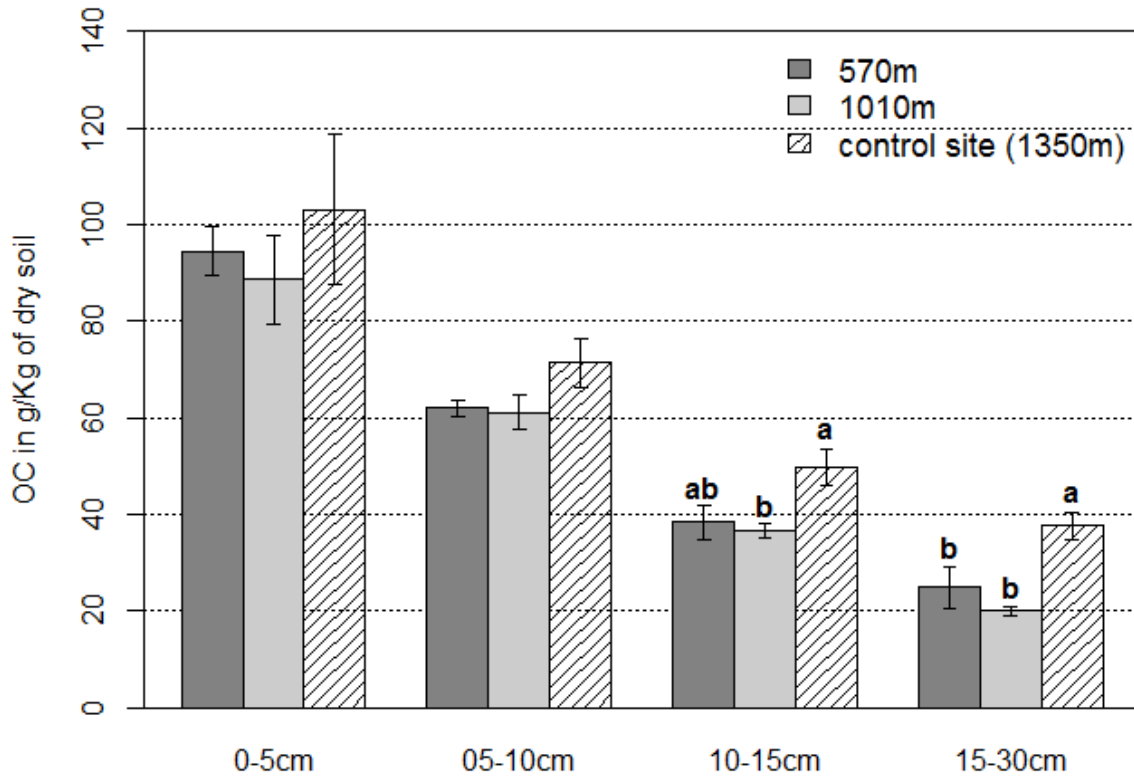
**Figure S1. Soil organic carbon content for the four soil depths sampled in summer 2014.** Error bars represent the standard errors of the mean values (n = 5). Lower case letters indicate significant differences between transplantation sites (climate manipulation) at each soil depth.
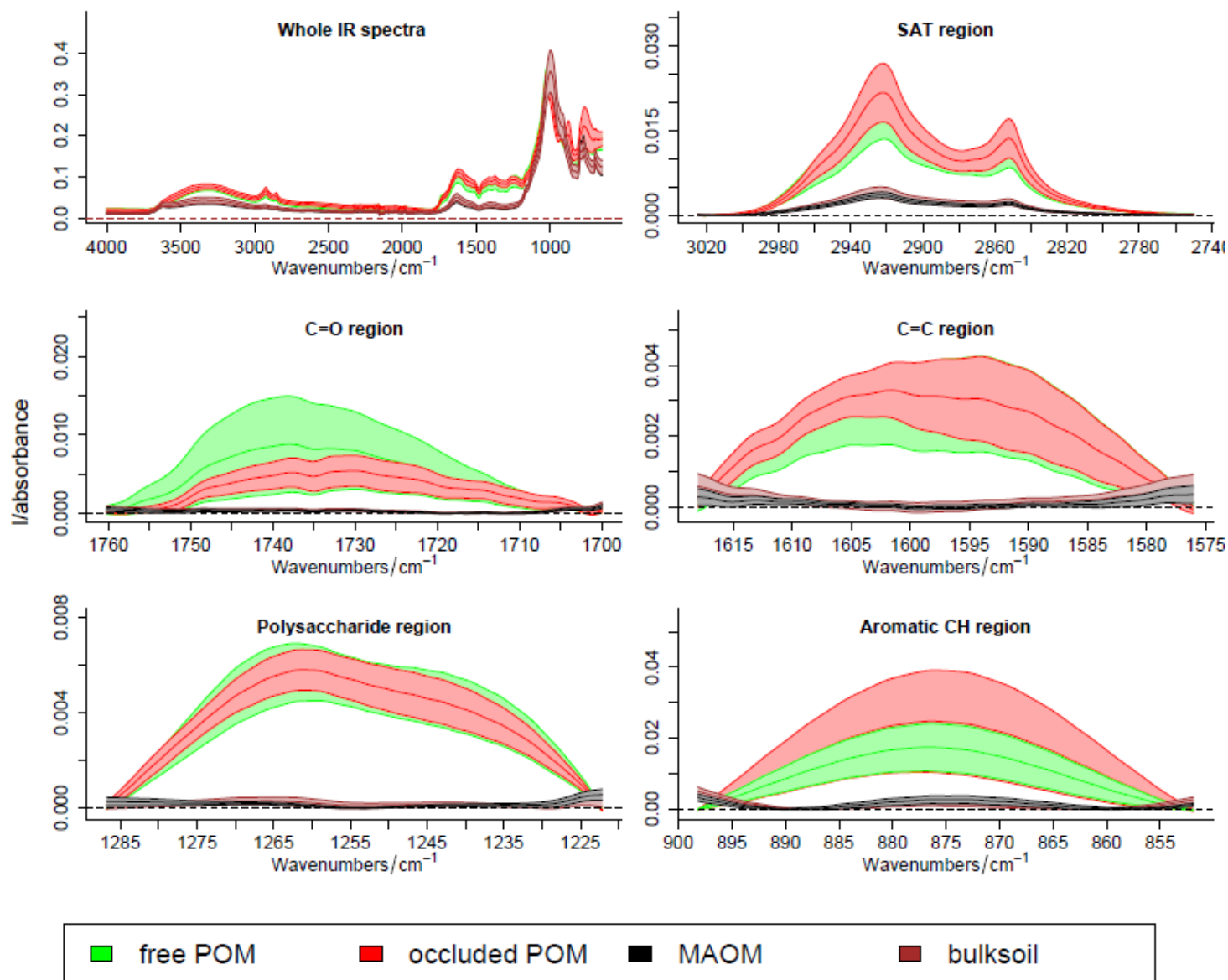
**Figure S2. Spectral regions of mid-infrared spectroscopy selected for building specific C functional indices.** The whole spectra chart indicates a highly different IR signal between POM fractions (free and occluded) and both MAOM fraction and bulk soil.

| Mixed models and P values | season F | season P | site F | site P | fraction F | fraction P | season:site F | season:site P | season:fraction F | season:fraction P | site:fraction F | site:fraction P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **IR indices (freePOM and occPOM)** | | | | | | | | | | | | |
| HI | 3.96 | 0.07 | 0.43 | 0.66 | 5.15 | * | 0.77 | 0.49 | 20.50 | *** | 1.92 | 0.17 |
| C=O/SOC | 19.38 | *** | 0.59 | 0.57 | 22.93 | *** | 1.49 | 0.26 | 28.26 | *** | 9.64 | *** |
| C=C/SOC | 3.89 | 0.07 | 0.08 | 0.92 | 2.46 | 0.13 | 1.45 | 0.27 | 13.98 | ** | 7.35 | ** |
| MI | 12.54 | ** | 0.12 | 0.89 | 12.54 | ** | 0.62 | 0.55 | 25.27 | *** | 10.39 | *** |
| PI | 9.63 | ** | 1.58 | 0.25 | 0.21 | 0.65 | 3.67 | 0.06 | 2.83 | 0.11 | 0.87 | 0.43 |
| CI | 7.43 | * | 1.66 | 0.23 | 0.05 | 0.83 | 0.93 | 0.42 | 48.55 | *** | 0.91 | 0.42 |
| Alkyl-C/Alkyl-O | 0.07 | 0.78 | 3.33 | 0.07 | 15.95 | *** | 2.76 | 0.08 | 29.13 | *** | 1.53 | 0.23 |
| **3D fluorescence indices (WEOC)** | | | | | | | | | | | | |
| Tyrosine/Humic | 94.70 | *** | 1.64 | 0.24 | --- | --- | 2.28 | 0.14 | --- | --- | --- | --- |
| Tyrosine /Fulvic | 49.70 | *** | 3.03 | 0.09 | --- | --- | 3.49 | 0.07 | --- | --- | --- | --- |
| Tryptophane/ Humic | 7.90 | * | 6.00 | * | --- | --- | 0.20 | 0.80 | --- | --- | --- | --- |
| Tryptophane / Fulvic | 0.00 | 0.99 | 11.03 | ** | --- | --- | 0.24 | 0.79 | --- | --- | --- | --- |
| Tyrosine / Tryptophane | 76.14 | *** | 1.80 | 0.20 | --- | --- | 3.20 | 0.07 | --- | --- | --- | --- |
| Humic / Fulvic | 70.03 | *** | 0.30 | 0.74 | --- | --- | 3.54 | 0.06 | --- | --- | --- | --- |

**Supplementary Table 1: Effects of sampling date, climate manipulation, fraction and interactions of these three factors on C-POM and WEOC bulk chemistry.** Definition of the IR indices: (1) SAT/SOC = hydrophobicity index (SAT = saturated hydrocarbons = Alkyl C, and SOC = soil organic carbon content); (2) C=O/SOC (index of oxidation);

(3) C=C/SOC (aromaticity index); (4) MI = C=C/UNSAT (maturation index) (5) POLY/SOC (polysaccharide content); (6) AROCH/C=C (condensation index = degree of condensation of aromatics), (7) SAT/POLY to estimate the Alkyl-C/Alkyl-O. Definition of the 3D Fluorescence indices: Tyrosine-like ($\lambda$exc/$\lambda$emi: 235/310), tryptophane-like (235;295 / 350), fulvic-like (235;295;310 /410) and humic-like (295; 310 /480) compounds. *Significance codes for ANOVA's are (\*\*\*) p<0.001; (\*\*) p<0.01; (\*) p<0.05.*