

- 1 **Lorenz, K., Lal, R., Preston, C. M., Nierop, K. G. J. 2007. Strengthening the soil**
- 2 **organic carbon pool by increasing contributions from recalcitrant aliphatic**
- 3 **bio(macro)molecules. Geoderma 142: 1-10.**

4 Strengthening the soil organic carbon pool by increasing contributions from recalcitrant
5 aliphatic bio(macro)molecules

6
7 Klaus Lorenz^{a,*}, Rattan Lal^a, Caroline M. Preston^b, Klaas G. J. Nierop^c

8 ^aCarbon Management and Sequestration Center, School of Environment and Natural
9 Resources, The Ohio State University, Columbus, Ohio, 43210, U.S.A.

10 ^bPacific Forestry Centre, Natural Resources Canada, Victoria BC, V8Z 1M5, Canada

11 ^cInstitute for Biodiversity and Ecosystem Dynamics, Earth Surface Processes and
12 Materials, Universiteit van Amsterdam, 1018 WV Amsterdam, The Netherlands

13

14 **Abstract**

15 Photosynthetically fixed CO₂ is converted into terrestrial bio(macro)molecules and
16 sequestered as soil organic matter (SOM) by (bio)chemical and physical stabilization
17 processes. SOM is generally divided in arbitrary pools for modeling SOM dynamics.
18 Biochemically recalcitrant SOM fractions are enriched with alkyl carbon (C) structures
19 and resist decomposition due to intrinsic molecular properties. The proportion of alkyl C
20 and the mean age of SOM increase with increase in soil depth. Precursors of these
21 recalcitrant bio(macro)molecules such as glycerides, waxes, and terpenoids occur in
22 plants, microorganisms and animals. The intrinsic biochemical stability of naturally
23 occurring recalcitrant aliphatic biomacromolecules may enhance the terrestrial storage of
24 atmospheric CO₂. Also, aliphatic macromolecules may be formed in soils upon non-
25 enzymatic polymerization of low-molecular-weight lipids. In this review we propose that

* Corresponding author. Fax: +1-614-292-7432

E-mail addresses: lorenz.59@osu.edu (K. Lorenz), lal.1@osu.edu (R. Lal), cpreston@pfc.cfs.nrcan.gc.ca (C. Preston), k.g.j.nierop@science.uva.nl (K. Nierop)

26 increasing the soil organic carbon (SOC) pool by land-use and management practices
27 should also include strategies to increase the proportion of aliphatic compounds in the
28 belowground biomass. Thus, collaborative research is needed to study the fate of plant-,
29 microbial- and animal-derived aliphatic C as precursors for stabilized aliphatic SOC
30 fractions, in particular in deeper soil horizons.

31

32 **Keywords:** Recalcitrant bio(macro)molecules; Alkyl carbon; Aliphatic compounds; Plant
33 lipids; Microbial lipids; Animal lipids; Soil carbon sequestration

34

35 **1. Introduction**

36

37 The lithosphere is the biggest C-reservoir (>75,000,000 gigatons (Gt) C), comprising
38 more than the oceans (>38,000 Gt C), and the terrestrial biosphere (>2,500 Gt C)
39 (Falkowski et al., 2000; Lal, 2004a). Atmospheric CO₂, however, exchanges rapidly on a
40 geological timescale only with reservoirs in terrestrial ecosystems and oceans.
41 Specifically, the primary producers (i.e., plants, cyanobacteria) remove CO₂ from the
42 atmospheric pool by photosynthesis, and fix C in a range of compounds constituting the
43 living biomass. Globally, only 4% to 6% of the 120 Gt C fixed annually remains in the
44 terrestrial biosphere (Schimel et al., 2001). In the terrestrial biosphere, the soil organic
45 carbon (SOC) pool is the largest (>1500 Gt C to 1-meter (m) or >2300 Gt C to 3-m)
46 (Jobbágy and Jackson, 2000). The processes governing stabilization of CO₂ into
47 biochemically recalcitrant biomacromolecules are, however, not fully known (Kramer
48 and Gleixner, 2006; von Lützow et al., 2006).

49 Biomacromolecules in soils are stabilized against biodegradation by selective
50 preservation due to biochemical recalcitrance and physical protection (Christensen,
51 1992). The chemical recalcitrance may be an inherent property of the molecular structure
52 (e.g., alkyl C chains in lipids, aromatic structures in aromatics and phenolics), but can
53 also be attained through formation of cross linkages between biomacromolecules which
54 inhibits enzymatic degradation, and by non-enzymatic polymerization of low-molecular-
55 weight compounds into macromolecules (Gleixner et al., 2001; de Leeuw et al., 2006).
56 Coating of organic matter (OM) with hydrophobic compounds that contain aliphatic
57 groups may contribute to SOC stabilization (Piccolo et al., 1999; Doerr et al., 2005).
58 Furthermore, spatially separating decomposers from biomacromolecules contributes to
59 SOC stabilization by entrapping organic compounds inside soil microaggregates within
60 macroaggregates (Six et al., 2002). Quantitative modeling approaches group SOM into
61 fractions with varying residence times, e.g., active (residence time 1 to 5 years), slow (20
62 to 40 years) and passive (400 to 2000 years) SOM fractions in the CENTURY model
63 (Parton et al., 1987). Furthermore, the proportion of the stable pool and the mean
64 residence time of SOC increase with increase in soil depth (Lorenz and Lal, 2005). In
65 view of the increasing atmospheric concentrations of CO₂, there is a strong need to
66 enhance our understanding of how terrestrial processes can be used to sequester
67 atmospheric CO₂ in biochemically recalcitrant compounds in particular in deeper soil
68 horizons.

69 Plant tissues and soil microorganisms are the primary sources of SOC (Figure 1) (Kögel-
70 Knabner, 2002; Kramer and Gleixner, 2006). Soil animals, on the other hand, contribute
71 less than 1% to the SOC pool but the C flux through the soil food web is not well

72 understood (Wolters, 2000; Osler and Sommerkorn, 2007). Major components of plant
73 tissues are polysaccharides, lignin, tannins and proteins, while soil bacteria and fungi
74 consist mainly of homo- and heteropolysaccharides (e.g., chitin, peptidoglycan,
75 lipopolysaccharides), and animals mainly of carbohydrates, proteins and lipids. During
76 decomposition, 10-20% of the biomass is transferred to stable SOC fractions, but only
77 1% of the biomass ultimately remains buried in sediments on geological timescales (de
78 Leeuw and Largeau, 1993, Hedges and Oades, 1997), so that large amounts of C are
79 returned to the atmosphere as CO₂.

80 Processes governing transformations of biomass compounds into stable long-term C
81 pools are poorly understood (Hedges et al., 2000). Principal unknowns are the biological,
82 chemical and physical transformation of complex OM in natural environments, and the
83 role of microorganisms and animals in these processes. Despite recent progress, the
84 detailed chemical composition of over half of all OM on Earth is not known (Kögel-
85 Knabner, 2000). The role of microbial and faunal processes in the preservation of
86 recalcitrant biomacromolecules, and their role in long-term accumulation of SOC are
87 actively-debated issues (Ekschmitt et al., 2005; Albers et al., 2006; Six et al., 2006;
88 Pollierer et al., 2007). However, SOC fractions derived from plant biomacromolecules
89 (e.g., cutin and suberin (Winkler et al., 2005)), from microbial processes (e.g., microbial
90 lipids (Nierop et al., 2005b)), and from soil animals (e.g., chitin and proteins (Ekschmitt
91 et al., 2005; Nierop et al., 2005a)) are commonly found in soils. Recalcitrant SOC
92 fractions, which may constitute 15-50% of the SOC pool, are the least dynamic organic C
93 pools in terrestrial ecosystems, and may be a very long-term CO₂ sink with estimated
94 ages of several hundred to several thousand years (Falloon and Smith, 2000).

95 Therefore, the objectives of this review are to: (i) describe biochemically recalcitrant
96 (macro)molecules or fractions in the pedosphere and their relationship to possible
97 precursor compounds in terrestrial plants, soil microorganisms and animals, and (ii)
98 propose management options for increasing the proportion of recalcitrant
99 biomacromolecules in deeper soil horizons. Reviews and hypotheses about other
100 mechanisms on transformation of bio(macro)molecules and their stabilization in SOM
101 can be found elsewhere (e.g., von Lützow et al., 2006; Kleber et al., 2007).

102

103 **2. Recalcitrant bio(macro)molecules**

104

105 *2.1. Precursor bio(macro)molecules of recalcitrant SOC*

106

107 The intrinsic resistance of biomacromolecules against biodegradation may partially be
108 related to essential supportive or protective functions (van Bergen et al., 2004). Table 1
109 gives an overview of potentially recalcitrant compounds in terrestrial plants, and
110 microbial and animal biomass (Killops and Killops, 2005). By comparing
111 biomacromolecule structures preserved in fossil fuels and fossil plant remains for
112 thousands to millions of years with those stored for thousands of years in sediments and
113 soil profiles, precursors in the terrestrial biomass with potentially high residence times
114 can be identified. Compounds resisting humification and, in particular, sedimentation and
115 diagenesis are therefore likely to contribute to long-term CO₂ sinks. Compounds in the
116 terrestrial plant biomass like cutans, cutins, glycolipids, lignins, resinous polyterpenoids,
117 sporopollenins, suberans, suberins and tannins have been proposed as potentially

118 recalcitrant (de Leeuw and Largeau, 1993; Kolattukudy, 2001; de Leeuw et al., 2006).
119 Glycolipids and the more recalcitrant lipopolysaccharides are part of the bacterial cell
120 membrane. Other identified potentially recalcitrant compounds are steranes derived from
121 regular steroids, and hopanes derived from the triterpenoid compounds hopanoids
122 (Gleixner et al., 2001, Killops and Killops, 2005).

123

124 *2.2. Fate of recalcitrant aliphatic compounds in the terrestrial environment*

125

126 In soils, only a few organic compounds have high intrinsic resistance to enzymatic attack
127 (Ekschmitt et al., 2005). Recalcitrant biomacromolecules occur only in small amounts in
128 living organisms, and are selectively enriched in the stable SOC pool during
129 biodegradation (Krull et al., 2003). Furthermore, recalcitrant macromolecules may be
130 formed by non-enzymatic polymerization of low-molecular-weight precursors (de Leeuw
131 et al., 2006). Even trace inputs of recalcitrant compounds and their precursor compounds
132 may therefore significantly contribute to the stable SOC pool (Hedges et al., 2000).

133 According to the older “humic” concept of SOM, recalcitrant lignin-derived aromatics
134 were the source of the main structural framework for humic substances, and thus, the
135 bulk of stabilized SOM (Stevenson, 1994). Humic substances may, however, not be a
136 distinct chemical category as modern multidimensional nuclear magnetic resonance
137 (NMR) approaches indicate (Kelleher and Simpson, 2006). Instead, the vast majority of
138 operationally defined soil humic material may be a very complex mixture of faunal,
139 microbial and plant biopolymers and their degradation products. Proteins, lignin,
140 carbohydrates and aliphatic biopolymers are the major components of the mixtures.

141 Otherwise, it was hypothesized previously that lignin is a recalcitrant biomacromolecule,
142 and thus should accumulate in SOM relative to other more labile compounds. Soil fungi,
143 however, decompose lignin, and actinomycetes and bacteria alter lignin in soil (Table 2;
144 de Boer et al., 2005; Dignac et al., 2005). Furthermore, the previously widely-used
145 proximate analysis for “lignin” (i.e., the acid-insoluble residue), originally developed for
146 wood chemistry and forage digestibility is not suitable to determine lignin as the residue
147 also contains other compounds, e.g., cutin and tannin (Preston et al., 1997; 2006). Further
148 evidence for the relative decomposability of lignin arises from the observation that lignin
149 phenols are not enriched in soil profiles relative to suberin and cutin compounds in
150 temperate and tropical organic and mineral (sub)soil horizons (Zech and Guggenberger,
151 1996; Nierop, 2001; Nierop and Verstraten, 2003; Rumpel et al., 2004). Thus, lignin is
152 not preserved in subsoil horizons in forest and grassland ecosystems (Rumpel et al.,
153 2004; Feng and Simpson, 2007). Compound-specific stable-isotope analyses indicate that
154 soil lignin consists of a rapid decomposing pool (turnover time <1 year, 93% of total
155 lignin) derived from fresh-litter lignin, and a slow decomposing pool of lignin stabilized
156 in SOM (turnover time 17 years, 7% of total lignin; Glaser, 2005; Rasse et al., 2006).
157 Thus, a relatively rapid turnover of lignin compounds in soil is likely (Nierop and Filley,
158 2007).

159 In contrast to lignin-derived aromatics, alkyl C visible by ^{13}C -NMR accumulates over the
160 time scale of soil formation (Baldock et al., 2004). Alkyl C visible by NMR is mainly due
161 to CH_2 units in alkyl chains and terminal CH_3 groups (Preston et al., 1997). Among the
162 few published ^{13}C -NMR studies of soil profiles, some reported higher contributions of
163 alkyl C to subsoil OM than to surface SOM (e.g., Beyer et al., 1992; Baldock et al., 1997;

164 Dai et al., 2001; Skjemstad et al., 2001; Rumpel et al., 2002; Ussiri and Johnson, 2003).
165 In grassland soil profiles, suberin biomarkers identified by chemolysis and gas
166 chromatography/mass spectrometry (GC/MS) increased with soil depth while cutin
167 biomarkers decreased (Feng and Simpson, 2007). In the subsoil, 17-47% of the identified
168 SOM consisted of suberin biomarkers, and suberin/cutin ratios in subsoils not affected by
169 eluvation were higher than those in surface horizons. In sandy subsoils, both extractable
170 and ester-bound lipids in the form of suberin increased upon acidification and age (10.2%
171 and 12.6% of SOM weight derived from extractable lipids and suberin, respectively)
172 (Nierop and Verstraten, 2003).

173 In ecosystems dominated by non-woody species such as grasslands, suberin is a good
174 tracer of root biomass (Rasse et al., 2005). Otherwise, in a forest profile which was also
175 not affected by eluvation bulk SOM was 1700 years older in 50-80 cm depth than in 0-5
176 cm depth (Rumpel et al., 2004). Furthermore, the percentage of alkyl C in the 0.2-2 μm
177 fraction increased from 39% in 0-5 cm depth to 48% in 50-80 cm depth. The fine
178 particle-size fractions in this soil profile were generally older than the bulk soil, and
179 cutin/suberin-derived hydroxyalkanoic acids were preserved in fine particles. In a study
180 of an Andosol catena, the relative accumulation of alkyl compounds in subsoils may have
181 been caused by higher contributions of suberin-containing root litter although
182 preferentially decay of other compounds may have also contributed to the relative
183 accumulation of aliphatics (Buurman et al., 2007). More aliphatics were found in
184 Ecuadorian Andosols, especially in subsoil horizons where SOM was ca. 2000 years old
185 (Nierop et al., 2007). In summary, inputs of suberin from root tissue to the subsoil where
186 SOC is stored for long periods of time are potential sinks of atmospheric CO₂.

187

188 *2.3. Formation of recalcitrant aliphatic soil organic matter fractions*

189

190 Alkyl C as in polymethylenic compounds is among the biologically most stable forms of
191 SOC in aerobic soils (von Lützow et al., 2006). In anaerobic sediments, aliphatic
192 biomacromolecules, beside lignin and black C, also contribute to OM (Dickens et al.,
193 2006). Fossil remains of recalcitrant aliphatic biomacromolecules are also frequently
194 observed (Nip et al., 1986; Möhle et al., 1998; Hedges et al., 2000). Studies have shown
195 recently that in the absence of resistant aliphatic precursors (e.g., cutan), relatively labile
196 alkyl C compounds can be the source of the insoluble aliphatic component of fossil OM
197 (Gupta et al., 2007). In addition to the selective preservation of resistant aliphatic
198 biomacromolecules (Gleixner et al., 2001; Krull et al., 2003), non-biological aliphatic
199 geomacromolecules can be formed in soils (de Leeuw et al., 2006). Specifically, highly
200 aliphatic and resistant geopolymers may be formed in soils upon oxidative
201 polymerization of low-molecular-weight membrane and other (presumably unsaturated)
202 lipids (de Leeuw, 2007). Thus, non-enzymatic processes may also contribute to the
203 formation of recalcitrant SOM fractions in the oxidative depositional soil environment.
204 Experimental evidence for processes governing the formation of aliphatic SOM indicate
205 that chemically-labile lipids are preserved in soils (Nierop et al., 2003). For example,
206 SOM in sandy soils with higher lipid and suberin contents was older according to
207 radiocarbon (^{14}C) dating models than SOM in sandy soils with lower lipid and suberin
208 contents (625 years B.P. vs. 'modern'; Nierop, unpublished results). Furthermore,
209 aliphatic hydrocarbons more than 10000 year old were found in upland soils, persistent

210 due to low biodegradability of long-chain *n*-alkanes (Huang et al., 1999). Recalcitrant
211 SOM fractions in forest subsoil horizons were older (505-3385 years B.P.) than bulk
212 SOM, and mainly composed of alkyl C whereas lignin phenols were present only in small
213 amounts (Mikutta et al., 2006). The importance of alkyl C relative to lignin for the slower
214 or stabilized SOM pool, however, so far has not been adequately addressed as many
215 models simulating SOM dynamics are still based on proximate analysis with acid-
216 insoluble residue incorrectly called “lignin” (e.g., Shibu et al., 2006).

217

218 **3. Management options to increase recalcitrant biomacromolecules in terrestrial** 219 **ecosystems**

220

221 *3.1. Plant species*

222

223 Highly aliphatic biomacromolecules derived from plant tissues may contribute stabilized
224 aliphatic hydrocarbons to SOM. Cutin is the major lipid polymer in plants making the
225 leaves water- and air-tight, and protecting the plant against microbial attack (Heredia,
226 2003). Furthermore the highly aliphatic and resistant biopolymer cutan makes leaf
227 cuticles watertight in drought-adapted CAM plants (Boom et al., 2005). Suberin
228 comprises both aliphatic and aromatic domains, and covers all belowground plant tissues
229 but occurs also in cork tissues, and bundle-sheath cells in C₄ plants making the cell wall
230 water- and air-tight (Bernards, 2002). Breeding and cultivating plant species rich in the
231 aliphatic biopolymers cutin, cutan and suberin is, therefore, a potential but not-yet-
232 studied option to increase proportions of recalcitrant biomacromolecules in terrestrial

233 ecosystems. Plants with a large amount of belowground parts will increase the suberin
234 concentration in the mineral soil. Breeding plants with high concentrations of aliphatic
235 biomacromolecules in root tissue may also strengthen cultivation of biomass as a carbon-
236 neutral renewable resource for the production of bioenergy and biomaterials (Ragauskas
237 et al., 2006).

238 One pre-requisite for promoting plant biomass rich in aliphatic biomacromolecules as C
239 sink are studies of the C transfer from these compounds to stable SOC fractions. For
240 example, the thickness, composition and structure of the plant cuticle may be criteria to
241 select appropriate cultivars for SOC sequestration as these properties vary widely among
242 plant species and tissues (Nawrath, 2006). Recently, phenotypes of *Arabidopsis* mutants
243 that have alterations in their cuticle have been characterized, and this information may
244 help to identify genes responsible for the regulation of the biosynthesis of cuticular
245 components. Transgenic plants with a defined altered cutin composition may then be
246 engineered. For example, the overexpression of certain genes in wax biosynthesis leads to
247 an increase in wax load and altered properties of the cuticular membrane in *Arabidopsis*
248 (Nawrath, 2006).

249 By contrast, knowledge about suberin biosynthesis is scanty, but a hypothetical pathway
250 for suberin and cutin biosynthesis in *Arabidopsis* was proposed by Franke et al. (2005).
251 Accordingly, the precursors for suberin and cutin are supplied by the pool of C₁₆ and C₁₈
252 carboxylic acids derived from fatty acid biosynthesis. The biosynthesis of long-chain
253 fatty acids of potato suberin follows a conventional pathway (Yan and Stark, 2000). Yet,
254 it needs to be tested if manipulation of suberin and cutin biosynthesis affects the
255 biosynthesis of other compounds sharing the same precursors. The acetate-malate

256 pathway leads via lipid-synthesis to cutin and cutan (de Leeuw et al., 2006). Storage and
257 membrane lipids are, however, also derived from fatty acids whereas isoprenoids and
258 terpenoids (Table 1) have the same precursors as fatty acids (i.e., acetyl CoA).
259 Data on concentrations of glycerides, waxes and terpenoids of plant tissues, especially for
260 crop residues would also help to foster the cultivation of plants as sources for recalcitrant
261 SOC fractions. Concentrations of biomacromolecules in forest and crop residues, and
262 their molecular composition indicated by relative intensities in solid-state ^{13}C NMR
263 spectroscopy vary considerably (Table 3) (Kögel-Knabner, 2002). Woody species tend to
264 have higher proportions of alkyl C (e.g., in bark) compared to agricultural species, and
265 plant roots have high proportions of alkyl C (Lorenz and Lal, 2005). Thus, woody plants
266 and/or plants with high root-to-shoot ratios are potential sources of recalcitrant
267 biomacromolecules in soils. The importance of root-derived C as primary source of SOC
268 due to inherent recalcitrance of root OM and root-induced stabilization mechanisms has,
269 however, received less attention than the importance of aboveground plant residues (Gale
270 et al., 2000; Puget and Drinkwater, 2001; Rasse et al., 2005). In particular, contributions
271 of roots to SOC in agricultural soils have not been extensively studied. The data base
272 even for the more comprehensive studied forest ecosystems is scanty. For example,
273 globally there are only 56 reports describing complete tree root profiles (Schenk and
274 Jackson, 2005).

275

276 3.2. *Soil microorganisms*

277

278 Relatively little is known about the metabolic capabilities and composition of most soil
279 microorganisms, largely because of their resistance to culture under standard laboratory
280 conditions (Six et al., 2006). Furthermore, little is known about the fate of the multitude
281 of microbial components in the soil (Kögel-Knabner, 2002). Recently, it was shown for
282 Andosols that microbial OM replaced plant-derived components in SOM at faster rates
283 than observed in other soil types (Nierop et al., 2005a; Buurman et al., 2007). Towards a
284 complete understanding of the SOC cycle, more studies incorporating microbial
285 processes are therefore urgently needed.

286 Archaea are ubiquitous in soils and transcriptionally active, but their total biomass in soil
287 is not known (Weijers et al., 2006). Archaea together with bacteria are the most
288 metabolically diverse of all soil microorganisms (Leininger et al., 2006). Membrane
289 lipids of archaea have recently been detected in a variety of globally-distributed soil
290 samples (Weijers et al., 2006). In archaeal lipids, branched-chain hydrocarbons are linked
291 to glycerol by ether bonds, and diether lipids and tetraethers containing long-chain
292 hydrocarbons are also found in archaea (van de Vossenberg, 1998). Ether lipids cannot
293 easily be degraded, and tetraether lipids occur in rigid membranes nearly impermeable to
294 ions and protons. Thus, archaeal ether and tetraether lipids are potential sources of the
295 recalcitrant SOC fraction.

296 Bacterial amino sugars may turn over in 3 to 90 years in soil but no literature is available
297 on the turnover of other bacterial compounds (Glaser et al., 2006). Bacteria produce
298 melanins, waxes, terpenoids, and tetrapyrrole pigments that may be biochemically
299 recalcitrant and resistant to biodegradation in soil (Gleixner et al., 2001).

300 The non-hydrolyzable melanins of fungi consist of proteins, carbohydrates, lipids, and a
301 polymer built from phenolic, indolic, quinone, hydro-quinone and semi-quinone
302 monomers (Kögel-Knabner, 2002). Melanins are highly resistant to decomposition and
303 considered biochemically recalcitrant due to their aromatic structure but structural
304 differences among them lead to great differences in their stability in soils (Martin et al.,
305 1982). Melanins protect fungal cell walls against microbial decomposition but their fate
306 in soil is not known (von Lützow et al., 2006). Sporopollenins are found in fungal spores
307 (Killops and Killops, 2005). They consist of p-coumaric acid units with some
308 contribution of ferulic acid (Boom, 2004), and exhibit high resistance under natural
309 conditions but the alteration of their chemical structure in soil has not been widely
310 studied (Derenne and Largeau, 2001). The steroid ergosterol is a highly specific
311 biomarker for fungi but its stability in soil remains to be investigated (Högberg, 2006).
312 For example, in contrast to previous assumptions of a rapid turnover, a half-life of
313 ergosterol in soil of ca. 3-5 months was reported (Mille-Lindblom et al., 2004).

314

315 *3.3. Animals*

316

317 Soil animals significantly affect SOC pools and fluxes (Osler and Sommerkorn, 2007).
318 Specifically, soil animal food webs seem to be mainly based on root exudates, litter and
319 recalcitrant SOM as C sources (Ruf et al., 2006). Living plants, however, most strongly
320 affect C flows through the food web. Thus, increasing plant cover as food source for
321 animals may increase the proportions of potentially recalcitrant bio(macro)molecules in
322 animal residues (Table 1). Faunal biomacromolecules with potential resistance to

323 decomposition are lipids and waxes. Chitin occurring in soil invertebrates and molluscs,
324 and, hardened by the protein sclerotin, in arthropod cuticles may also be a recalcitrant
325 compound (Briggs et al., 1998; Wolters, 2000). Although arthropods contain no
326 biochemically-recalcitrant aliphatic compounds, *in situ* polymerization probably
327 preserved aliphatic components in arthropod fossils, but the importance of this process
328 for soil fauna decomposition needs to be investigated (Gupta et al., 2007). Chitin may
329 also be gradually replaced by an aliphatic geopolymer produced from lipids upon
330 oxidative polymerization (Gupta et al., 2006). In temperate mineral soils, however, chitin
331 is almost completely recycled but probably not in volcanic ash soils (Gooday, 1990;
332 Nierop et al., 2005a; Buurman et al., 2007).

333 Highly-ordered and cross-linked faunal proteins such as keratin, fibrinogen and collagen
334 are less accessible to extracellular enzymes and may resist decomposition in soil, in
335 particular when they are located within a mineral matrix such as bone (Ekschmitt et al.,
336 2005; Killops and Killops, 2005). In particular collagen contributes to protein in human
337 remains preserved in peat bogs by tanning reaction with sphagnum (Painter, 1991;
338 Stankiewicz et al., 1997). Besides directly increasing recalcitrant bio(macro)molecules
339 from animal growth by increased plant cover, animal metabolism may alter recalcitrant
340 bio(macro)molecules in plant residues. For example, during passage through the animal
341 gut, litter may lose sterols, short-chain fatty acids, triacylglycerols, amino acids and
342 polysaccharides but accumulate triterpenoids, wax esters and aromatic-C (Hopkins et al.,
343 1998; Rawlins et al., 2006). However, C transfer from stable to more labile SOC pools
344 during gut passage is also observed (Fox et al., 2006). Soil animals indirectly alter the
345 chemical composition of litter through their grazing activity on bacteria and fungi

346 (Chapin et al., 2002). The major effect of the soil fauna on chemical alteration of litter
347 and SOM occurs, however, through enhancement of microbial activity by fragmentation
348 (Wolters, 2000). Furthermore, predators may indirectly cause the accumulation of total
349 phenolics in leaf litter but the loss of cellulose and condensed tannins (Hunter et al.,
350 2003).

351

352 *3.4. Soil management*

353

354 Soil management practices which increase the SOC pool (i.e., by SOC sequestration)
355 may also increase the proportion of recalcitrant biomacromolecules because these are
356 selectively enriched during biodegradation and recalcitrant geomacromolecules are
357 produced upon polymerization from low-molecular-weight membrane and other lipids
358 (Krull et al., 2003; Lal, 2004a; de Leeuw et al., 2006; de Leeuw, 2007). Therefore, the
359 recalcitrant SOC pool may be increased by increasing OM inputs into the ecosystem by
360 growing appropriate species, adopting recommended management practices, returning
361 residues to the soil (e.g., mulch farming, during forest harvesting and site preparation),
362 maintaining a continuous vegetation cover, growing cover crops during the off-season,
363 and applying manure, compost and biosolids (Lal, 2004b; Lal, 2005). For example, forest
364 residues (e.g., bark, roots) are high in lipids and alkyl C, and should be left on the soil for
365 biodegradation at the forest site (Table 3). Suppressing biodegradation and controlling
366 erosion may also reduce net SOC losses, and enhance the terrestrial sink of atmospheric
367 CO₂ in recalcitrant SOC pools. This may be accomplished by adopting conservation
368 tillage, agroforestry, diverse cropping systems, controlled grazing, afforestation and

369 restoration of degraded soils and ecosystems (Lal, 2004b). Soil microorganisms and
370 animals benefit from increase in plant biomass inputs, and increase in SOC
371 concentrations through adoption of soil management strategies (Kramer and Gleixner,
372 2006). Thus, the proportion of recalcitrant (i.e., highly aliphatic) biomacromolecules in
373 the SOC pool derived from microorganisms and animals may also increase (Lorenz and
374 Lal, 2005).

375

376 *3.5. Saturation of the recalcitrant soil organic carbon pool*

377

378 The storage capacity of the SOC pool depends on the physicochemical characteristics
379 inherent to soils and the formation of biochemically stabilized SOM fractions (Six et al.,
380 2002). However, a mechanistic understanding governing transfer of C to the
381 biochemically recalcitrant SOC pool is still at an elementary level. Furthermore, the
382 maximum protective capacity for the recalcitrant pool may be defined by inherent soil
383 properties but it is not known if increasing biochemically-recalcitrant organic residue
384 input can be used to manipulate the size of this pool. That the transient state of SOC
385 pools is not adequately accounted for in SOM models was recently highlighted by
386 Wutzler and Reichstein (2007). In particular, very old soils still accumulate carbon
387 because of slowly ongoing accumulation of the slowest pool. The size of the recalcitrant
388 SOC pool in subsoil horizons can probably also be manipulated, as a decadal-reactive
389 SOC pool below 50-cm depth was recently observed in grassland soil profiles (Baisden
390 and Parfitt, 2007). This deep, reactive SOC may react to land-use or vegetation changes,
391 and respond to different processes than the reactive SOC in overlying horizons. Yet, it

392 needs to be tested if through increasing input of recalcitrant aliphatic C derived from
393 plants, microorganisms and animals, and through increasing input of lipid precursors for
394 non-enzymatic polymerization processes, a higher proportion of the recalcitrant in
395 relation to the total SOC pool can be achieved in particular in the subsoil.

396

397 **4. Conclusions**

398

399 Fossil fuel use will continue to increase for several decades (Lincoln, 2005). At least until
400 CO₂ emission reduction technologies take effect, terrestrial sequestration of CO₂ will
401 remain to be an important strategy. Due to considerable importance for SOC
402 sequestration, it needs to be studied in detail whether increases in radiocarbon age with
403 depth correlate with increases in the proportion of recalcitrant SOC, and if land-use and
404 soil management can be used to increase this fraction. The adoption of recommended
405 land-use and soil management techniques to increase the SOC pool may be accompanied
406 by increasing contributions of plant-derived aliphatic compounds (e.g., cutin, cutan,
407 suberin) and aliphatic compounds derived from microorganisms and animals, especially
408 in deeper soil horizons. Traditionally, forest and agricultural land-uses are primarily
409 aimed at maximizing the aboveground growth. For promoting the cultivation of plants
410 which increase and stabilize the SOC pool, the data base on the composition of organic
411 resources must be strengthened by including data for aliphatic bio(macro)molecules
412 (Palm et al., 2001). The biological flow of C through soil is a research priority (Janzen,
413 2006), and studies on the fate of C derived from aliphatic compounds in recalcitrant SOC
414 fractions are needed. Recalcitrant compounds are much more abundant in soil than easily

415 degradable compounds but effects of climate change on their turnover are unknown
416 (Davidson and Janssens, 2006). More information is required on the origin of alkyl C
417 compounds in soils and the involved mechanisms, e.g, the proportion of aliphatic
418 compounds derived from plants that is transformed by the microbial and animal
419 metabolism vs. the proportion subject to non-enzymatic polymerization before being
420 stored in aliphatic SOC fractions. Transformations of plant, microbial and animal alkyl
421 (and other) C can be studied by compound-specific techniques, but these can only relate
422 to compounds that can still be identified. Interdisciplinary studies, comprising plant
423 biochemists, microbiologists and organic geochemists, are needed to manage the intrinsic
424 chemical stability of naturally occurring recalcitrant bio(macro)molecules and their
425 formation to enhance the terrestrial sequestration of atmospheric CO₂.

426

427 **References**

428

429 Albers, D., Schaefer, M., Scheu, S., 2006. Incorporation of plant carbon into the soil
430 animal food web of an arable system. *Ecology* 87: 235-245.

431 Baisden, W.T., Parfitt, R.L., 2007. Bomb ¹⁴C enrichment indicates decadal C pool in
432 deep soil? *Biogeochemistry* 85: 59-68.

433 Baldock, J.A., Oades, J.M., Nelson, P.N., Skene, T.M., Golchin, A., Clarke, P., 1997.
434 Assessing the extent of decomposition of natural organic materials using solid-state ¹³C
435 NMR spectroscopy. *Aus. J. Soil Res.* 35: 1061-1083.

436 Baldock, J.A., Masiello, C.A., Gélinas, Y., Hedges, J.I., 2004. Cycling and composition
437 of organic matter in terrestrial and marine ecosystems. *Mar. Chem.* 92: 39-64.

438 Bernards, M.A., 2002. Demystifying suberin. *Can. J. Bot.* 80: 227-240.

439 Beyer, L., Schulten, H.-R., Fründ, R., 1992. Properties and composition of soil organic
440 matter in forest and arable soils of Schleswig-Holstein: 1. Comparison of morphology
441 and results of wet chemistry, CPMAS-¹³C-NMR spectroscopy and pyrolysis-field
442 ionization mass spectrometry. *Z. Pflanzenernähr. Bodenk.* 155: 345-354.

443 de Boer, W., Folman, L.B., Summerbell, R.C., Boddy, L., 2005. Living in a fungal world:
444 impact of fungi on soil bacterial niche development. *FEMS Microbiol. Rev.* 29: 795-811.

445 Boom, A., 2004. A geochemical study of lacustrine sediments: towards palaeo-climatic
446 reconstructions of high Andean biomes in Colombia. PhD Thesis University of
447 Amsterdam, 125 pp.

448 Boom, A., Sinnige Damsté, J.S., de Leeuw, J.W., 2005. Cutan, a common aliphatic
449 biopolymer in cuticles of drought-adapted plants. *Org. Geochem.* 36: 595-601.

450 Briggs, D.E.G., Evershed, R.P., Stankiewicz, B.A., 1998. The molecular preservation of
451 fossil arthropod cuticles. *Ancient Biomolecules* 2: 135-146.

452 Burke, R.A., Molina, M., Cox, J.E., Osher, L.J., Piccolo, M.C., 2003. Stable carbon
453 isotope ratio and composition of microbial fatty acids in tropical soils. *J. Environ. Qual.*
454 32: 198-260.

455 Burman, P., Peterse, F., Almendros Martin, G., 2007. Soil organic matter chemistry in
456 allophanic soils: a pyrolysis-GC/MS study of a Costa Rican Andosol catena. *Eur. J. Soil*
457 *Sci.* doi: 10.1111/j.1365-2389.2007.00925.x

458 Chapin, F.S., Matson, P.A., Mooney, H.A., 2002. Principles of terrestrial ecosystem
459 ecology. Springer, New York.

460 Christensen, B.T., 1992. Physical fractionation of soil organic matter in primary particles
461 and density separates. *Adv. Soil Sci.* 20: 2-90.

462 Dai, K.H., Johnson, C.E., Driscoll, C.T., 2001. Organic matter chemistry and dynamics in
463 clear-cut and unmanaged hardwood forest ecosystems. *Biogeochemistry* 54: 51-83.

464 Davidson, E.A., Janssens, I.A., 2006. Temperature sensitivity of soil carbon
465 decomposition and feedbacks to climate change. *Nature* 440: 165-173.

466 de Leeuw, J.W., 2007. On the origin of sedimentary aliphatic macromolecules: A
467 comment on recent publications by Gupta et al. *Org. Geochem.*
468 doi:10.1016/j.orggeochem.2007.05.010

469 de Leeuw, J.W., Largeau, C., 1993. A review of macromolecular organic compounds that
470 comprise living organisms and their role in kerogen, coal, and petroleum formation. In:
471 M.H. Engel and S.A. Macko (Editors), *Organic Geochemistry*. Plenum Press, New York,
472 pp. 23-72.

473 de Leeuw, J.W., Versteegh, G.J.M., van Bergen, P.F., 2006. Biomacromolecules of algae
474 and plants and their fossil analogues. *Plant Ecol.* 182: 20-233.

475 Derenne, S., Largeau, C., 2001. A review of some important families of refractory
476 macromolecules: composition, origin, and fate in soils and sediments. *Soil Sci.* 166: 833-
477 847.

478 Dickens, A.F., Baldock, J.A., Smernik, R.J., Wakeham, S.G., Arnarson, T.S., Gélinas, Y.,
479 Hedges, J.I., 2006. Solid-state ^{13}C NMR analysis of size and density fractions of marine
480 sediments: Insight into organic carbon sources and preservation mechanisms. *Geochim.*
481 *Cosmochim. Ac.* 70: 666-686.

482 Dignac, M.-F., Bahri, H., Rumpel, C., Rasse, D.P., Bardoux, G., Balesdent, J., Girardin,
483 C., Chenu, C., Mariotti, A., 2005. Carbon-13 natural abundance as a tool to study the
484 dynamics of lignin monomers in soil: an appraisal at the Cloiseau experimental field
485 (France). *Geoderma* 128: 3-17.

486 Doerr, S.H., Llewellyn, C.T., Douglas, P., Morley, C.P., Mainwaring, K.A., Haskins, C.,
487 Johnsey, L., Ritsema, C.J., Stagnitti, F., Allinson, G., Ferreira, A.J.D., Keizer, J.J.,
488 Ziogas, A.K., Diamantis, J., 2005. Extraction of compounds associated with water
489 repellency in sandy soils of different origin. *Austr. J. Soil Res.* 43: 225-237.

490 Ekschmitt, K., Liu, M., Vetter, S., Fox, O., Wolters, V., 2005. Strategies used by soil
491 biota to overcome soil organic matter stability – why is dead organic matter left over in
492 the soil? *Geoderma* 128: 167-176.

493 Falkowski, P., Scholes, R.J., Boyle, E., Canadell, J., Canfield, D., Elser, J., Gruber, N.,
494 Hibbard, K., Högberg, P., Linder, S., Mackenzie, F.T., Moore, III B., Pedersen, T.,
495 Rosenthal, Y., Seitzinger, S., Smetacek, V., Steffen, W., 2000. The global carbon cycle: a
496 test of our knowledge of earth as a system. *Science* 290: 291-296.

497 Falloon, P.D., Smith, P., 2000. Modelling refractory soil organic matter. *Biol. Fertil.*
498 *Soils* 30: 388-398.

499 Feng, X., Simpson, M.J., 2007. The distribution and degradation of biomarkers in Alberta
500 grassland soil profiles. *Org. Geochem.* doi:10.1016/j.orggeochem.2007.05.001

501 Franke, R., Briesen, I., Wojciechowski, T., Faust, A., Yephremov, A., Nawrath, C.,
502 Schreiber, L., 2005. Apoplastic polyesters in *Arabidopsis* surface tissues - A typical
503 suberin and a particular cutin. *Phytochemistry* 66: 2643-2658.

504 Gale, W.J., Cambardella, C.A., 2000. Carbon dynamics of surface residue- and root-
505 derived organic matter under simulated no-till. *Soil Sci. Soc. Am. J.* 64: 190-195.

506 Glaser, G., 2005. Compound-specific stable-isotope ($\delta^{13}\text{C}$) analysis in soil science. *J.*
507 *Plant Nutr. Soil Sci.* 168: 633-648.

508 Glaser, B., Millar, N., Blum, H., 2006. Sequestration and turnover of bacterial and
509 fungal-derived carbon in a temperate grassland soil under long-term elevated atmospheric
510 pCO_2 . *Glob. Change Biol.* 12: 1521-1531.

511 Gleixner, G., Czimczik, C.J., Kramer, C., Lühker, B., Schmidt, M.W.I., 2001. Plant
512 compounds and their turnover and stabilization as soil organic matter. In: E.-D. Schulze,
513 M. Heimann, S. Harrison, E. Holland, J. Lloyd, I.C. Prentice and D. Schimel (Editors),
514 *Global Biogeochemical Cycles in the Climate System*. Academic Press, San Diego, pp.
515 201-215.

516 Gleixner, G., Poirier, N., Bol, R., Balesdent, J., 2002. Molecular dynamics of organic
517 matter in a cultivated soil. *Org. Geochem.* 33: 357-366.

518 Gooday, G.W., 1990. The ecology of chitin degradation. *Adv. Microbial Ecol.* 11: 387-
519 430.

520 Gregorich, E.G., Monreal, C.M., Schnitzer, M., Schulten, H.-R., 1996. Transformation of
521 plant residues into soil organic matter: chemical characterization of plant tissue, isolated
522 soil fractions, and whole soils. *Soil Sci.* 161: 680-693.

523 Gupta, N.S., Michels, R., Briggs, D.E.G., Collinson, M.E., Evershed, R.P., Pancost, R.D.,
524 2007. Experimental evidence for the formation of geomacromolecules from plant leaf
525 lipids. *Org. Geochem.* 38: 28-36.

526 Hedges, J.I., Oades, J.M., 1997. Comparative organic geochemistries of soils and marine
527 sediments. *Org. Geochem.* 27: 319-361.

528 Hedges, J.I., Eglinton, G., Hatcher, P.G., Kirchman, D.L., Arnosti, C., Derenne, S.,
529 Evershed, R.P., Kögel-Knabner, I., de Leeuw, J.W., Littke, R., Michaelis, W., Rullkötter,
530 J., 2000. The molecularly-uncharacterized component of nonliving organic matter in
531 natural environments. *Org. Geochem.* 31: 945-958.

532 Helfrich, M., Ludwig, B., Buurman, P., Flessa, H., 2006. Effect of land use on the
533 composition of soil organic matter in density and aggregate fractions as revealed by
534 solid-state ¹³C NMR spectroscopy. *Geoderma* 136: 331-341.

535 Heredia, A., 2003. Biophysical and biochemical characteristics of cutin, a plant barrier
536 biopolymer. *Biochim. Biophys. Acta* 1620: 1-7.

537 Högberg, M.N., 2006. Discrepancies between ergosterol and the phospholipid fatty acid
538 18:2ω6,9 as biomarkers for fungi in boreal forest soils. *Soil Biol. Biochem.* 38: 3431-
539 3435.

540 Hopkins, D.W., Chudek, J.A., Bignell, D.E., Frouz, J., Webster, E.A., Lawson, T., 1998.
541 Application of ¹³C NMR to investigate the transformations and biodegradation of organic
542 materials by wood- and soil-feeding termites, and a coprophagus litter-dwelling dipteran
543 larva. *Biodegradation* 9: 423-431.

544 Huang, Y., Li, B., Bryant, C., Bol, R., Eglinton, G., 1999. Radiocarbon dating of aliphatic
545 hydrocarbons: a new approach for dating passive-fraction carbon in soil horizons. *Soil*
546 *Sci. Soc. Am. J.* 63: 1181-1187.

547 Hunter, M.D., Adl, S., Pringle, C.M., Coleman, D.C., 2003. Relative effects of
548 macroinvertebrates and habitat on the chemistry of litter during decomposition.
549 *Pedobiologia* 47: 101-115.

550 Janzen, H.H., 2006. The soil carbon dilemma: Shall we hoard it or use it? *Soil Biol.*
551 *Biochem.* 38: 419-424.

552 Jobbágy, E.G., Jackson, R.B., 2000. The vertical distribution of soil organic carbon and
553 its relation to climate and vegetation. *Ecol. Appl.* 10: 423-436.

554 Kelleher, B.P., Simpson, A.J., 2006. Humic substances in soils: are they really
555 chemically distinct? *Environ. Sci. Technol.* 40: 4605-4611.

556 Killops, S., Killops, V., 2005. *Introduction to Organic Geochemistry*. Blackwell
557 Publishing, Malden (MA).

558 Kleber, M., Sollins, P., Sutton, R., 2007. A conceptual model of organo-mineral
559 associations in soils: self-assembly of organic molecular fragments into zonal structures
560 on mineral surfaces. *Biogeochemistry* 85: 9-24.

561 Kögel-Knabner, I., 2000. Analytical approaches for characterizing soil organic matter.
562 *Org. Geochem.* 31: 609-625.

563 Kögel-Knabner, I., 2002. The macromolecular organic composition of plant and
564 microbial residues as inputs to soil organic matter. *Soil Biol. Biochem.* 34: 139-162.

565 Kolattukudy, P.E., 2001. Polyesters in higher plants. In: Th. Scheper (Editor), *Advances*
566 *in Biochemical Engineering/Biotechnology*, vol 71. Springer-Verlag, Berlin, pp. 1-49.

567 Kramer, C., Gleixner, G., 2006. Variable use of plant- and soil-derived carbon by
568 microorganisms in agricultural soils. *Soil Biol. Biochem.* 38: 3267-3278.

569 Krull, E.S., Baldock, J.A., Skjemstad, J.O., 2003. Importance of mechanisms and
570 processes of the stabilisation of soil organic matter for modelling carbon turnover. *Funct.*
571 *Plant Biol.* 30: 207-222.

572 Lal, R., 2004a. Soil carbon sequestration impacts on global climate change and food
573 security. *Science* 304: 1623-1627.

574 Lal, R., 2004b. Soil carbon sequestration to mitigate climate change. *Geoderma* 123: 1-
575 22.

576 Lal, R., 2005. Forest soils and carbon sequestration. *Forest Ecol. Manage.* 220: 242-258.

577 Leininger, S., Urich, T., Schloter, M., Schwark, L., Qi, J., Nicol, G.W., Prosser, J.I.,
578 Schuster, S.C., Schleper, C., 2006. Archaea predominate among ammonia-oxidizing
579 prokaryotes in soils. *Nature* 442: 806-809.

580 Lincoln, S.F., 2005. Fossil fuels in the 21st century. *Ambio* 34: 621-627.

581 Lorenz, K., Preston, C.M., Krumrei, S., Feger, K.-H., 2004. Decomposition of needle/leaf
582 litter from Scots pine, black cherry, common oak and European beech at a conurbation
583 forest site. *Eur. J. For. Res.* 123: 177-188.

584 Lorenz, K., Lal, R., 2005. The depth distribution of soil organic carbon in relation to land
585 use and management and the potential of carbon sequestration in subsoil horizons. *Adv.*
586 *Agron.* 88: 35-66.

587 von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G.,
588 Marschner, B., Flessa, H., 2006. Stabilization of organic matter in temperate soils:
589 mechanisms and their relevance under different soil conditions – a review. *Eur. J. Soil*
590 *Sci.* 57: 426-445.

591 Madigan, M.T., Martinko, J.M., 2006. Brock – biology of microorganisms. Prentice Hall,
592 Upper Saddle River, NJ.

593 Mahieu, N., Powlson, D.S., Randall, E.W., 1999. Statistical analysis of published carbon-
594 ¹³C PMAS NMR spectra of soil organic matter. Soil Sci. Soc. Am. J. 63: 307-319.

595 Martin, J.P., Zunino, H., Peirano, P., Caiozzi, M., Haider, K., 1982. Decomposition of
596 ¹⁴C-labeled lignins, model humic acid polymers, and fungal melanins in allophanic soils.
597 Soil Biol. Biochem. 14: 289-293.

598 Matzke, K., Riederer, M., 1991. A comparative study into the chemical constitution of
599 cutins and suberins from *Picea abies* (L.) Karst., *Quercus robur* L., and *Fagus sylvatica*
600 L. Planta 185: 233-245.

601 Mikutta, R., Kleber, M., Torn, M.S., Jahn, R., 2006. Stabilization of soil organic matter:
602 association with minerals or chemical recalcitrance? Biogeochemistry 77: 25-56.

603 Mille-Lindblom, C., von Wachenfeldt, E., Tranvik, L.J., 2004. Ergosterol as a measure of
604 living fungal biomass: persistence in environmental samples after fungal death. J.
605 Microbiol. Meth. 59: 253-262.

606 Möhle, B., Collinson, M.E., Finch, P., Stankiewicz, B.A., Scott, A.C., Wilson, R., 1998.
607 Factors influencing the preservation of plant cuticles: a comparison of morphology and
608 chemical composition of modern and fossil samples. Org. Geochem. 29: 1369-1370.

609 Nawrath, C., 2006. Unraveling the complex network of cuticular structure and function.
610 Curr. Opin. Plant Biol. 9: 281-287.

611 Nierop, K.G.J., 2001. Temporal and vertical organic matter differentiation along a
612 vegetation succession as revealed by pyrolysis and thermally assisted hydrolysis and
613 methylation. J. Anal. Appl. Pyrolysis 61: 111-132.

614 Nierop, K.G.J., Verstraten, J.M., 2003. Organic matter formation in sandy subsurface
615 horizons of Dutch coastal dunes in relation to soil acidification. *Org. Geochem.* 34: 499-
616 513.

617 Nierop, K.G.J., Filley, T.R., 2007. Assessment of lignin and (poly-)phenol
618 transformations in oak (*Quercus robur*) dominated soils by ¹³C-TMAH
619 thermochemolysis. *Org. Geochem.* 38: 551-565.

620 Nierop, K.G.J., van Bergen, P.F., Buurman, P., van Lagen, B., 2005a. NaOH and
621 Na₄P₂O₇ extractable organic matter in two allophanic volcanic ash soils of the Azores
622 Islands – a pyrolysis GC/MS study. *Geoderma* 127: 36-51.

623 Nierop, K.G.J., Naafs, D.F.W., van Bergen, P.F., 2005b. Origin, occurrence and fate of
624 extractable lipids in Dutch coastal dune soils along a pH gradient. *Org. Geochem.* 36:
625 555-566.

626 Nierop, K.G.J., Naafs, D.F.W., Verstraten, J.M., 2003. Occurrence and distribution of
627 ester-bound lipids in Dutch coastal dune soils along a pH gradient. *Org. Geochem.* 34:
628 719-729.

629 Nierop, K.G.J., Tonneijck, F.H., Jansen, B., Verstraten, J.M., 2007. Organic matter in
630 volcanic ash soils under forest and Páramo along an Ecuadorian altitudinal transect. *Soil*
631 *Sci. Soc. Am. J* 71: 1119-1127.

632 Nip, M., Tegelaar, E.W., Brinkhuis, H., de Leeuw, J.W., Schenck, P.A., Holloway, P.J.,
633 1986. Analysis of modern and fossil plant cuticles by Curie point Py-GC and Curie point
634 Py-GC-MS: Recognition of a new, highly aliphatic and resistant biopolymer. *Org.*
635 *Geochem.* 10: 769-778.

636 Osler, G.H.R., Sommerkorn, M., 2007. Toward a complete soil C and N cycle:
637 incorporating the soil fauna. *Ecology* 88: 1611-1621.

638 Painter, T.J., 1991. Lindow man, Tollund man and other peat-bog bodies: the preservative
639 and antimicrobial action of sphagnan, a reactive glycuronoglycan with tanning and
640 sequestering properties. *Carbohydr. Polym.* 15: 123-142.

641 Palm, C.A., Gachengo, C.N., Delve, R.J., Cadisch, G., Giller, K.E., 2001. Organic inputs
642 for soil fertility management in tropical agroecosystems: application of an organic
643 resource database. *Agr. Ecosyst. Environ.* 83: 27-42.

644 Parton, W.J., Schimel, D.S., Cole, C.V., Ojima, D.S., 1987. Analysis of factors
645 controlling soil organic matter levels in Great Plain grasslands. *Soil Sci. Soc. Am. J.* 51:
646 1173-1179.

647 Piccolo, A., Spaccini, R., Haberhauer, G., Gerzabek, M.H., 1999. Increased sequestration
648 of organic carbon in soil by hydrophobic protection. *Naturwissenschaften* 86: 496-499.

649 Pollierer, M.M., Langel, R., Körner, C., Maraun, M., Scheu, S., 2007. The
650 underestimated importance of belowground carbon input for forest soil animal food webs.
651 *Ecol. Lett.* 10: 729-736.

652 Preston, C.M., Trofymow, J.A., Sayer, B.G., Niu, J., 1997. ^{13}C nuclear magnetic
653 resonance spectroscopy with cross-polarization and magic-angle spinning investigation of
654 the proximate-analysis fractions used to assess litter quality in decomposition studies.
655 *Can. J. Bot.* 75: 1601-1613.

656 Preston, C.M., Trofymow, J.A., Flanagan, L.B., 2006. Decomposition, $\delta^{13}\text{C}$, and the
657 'lignin paradox'. *Can. J. Soil Sci.* 86: 235-245.

658 Puget, P., Drinkwater, L.E., 2001. Short-term dynamics of root- and shoot-derived carbon
659 from a leguminous green manure. *Soil Sci. Soc. Am. J.* 65: 771-779.

660 Ragauskas, A.J., Williams, C.K., Davison, B.H., Britovsek, G., Cairney, J., Eckert, C.A.,
661 Frederick, Jr. W.J., Hallett, J.P., Leak, D.J., Liotta, C.L., Mielenz, J.R., Murphy, R.,
662 Templer, R., Tschaplinski, T., 2006. The path forward for biofuels and biomaterials.
663 *Science* 311: 484-489.

664 Rasse, D.P., Rumpel, C., Dignac, M.-F., 2005. Is soil carbon mostly root carbon?
665 Mechanisms for a specific stabilization. *Plant Soil* 269: 341-356.

666 Rasse, D.P., Dignac, M.-F., Bahri, H., Rumpel, C., Mariotti, A., Chenu, C., 2006. Lignin
667 turnover in an agricultural field: from plant residues to soil-protected fractions. *Eur. J.*
668 *Soil Sci.* 57: 530-538.

669 Rawlins, A.J., Bull, I.D., Poirier, N., Ineson, P., Evershed, R.P., 2006. The biochemical
670 transformation of oak (*Quercus robur*) leaf litter consumed by the pill millipede
671 (*Glomeris marginata*). *Soil Biol. Biochem.* 38: 1063-1076.

672 Rillig, M.C., Caldwell, B.A., Wösten, H.A.B., Sollins, P., 2007. Role of proteins in soil
673 carbon and nitrogen storage: controls on persistence. *Biogeochemistry* 85: 25-44.

674 Rosenberg, W., Nierop, K.G.J., Knicker, H., De Jager, P.A., Kreutzer, K., Weiß, T.,
675 2003. Liming effects on the chemical composition of the organic surface layer of a
676 mature Norway spruce stand (*Picea abies* [L.] Karst.). *Soil Biol. Biochem.* 35: 155-165.

677 Rowell, D.M., Prescott, C.E., Preston, C.M., 2001. Decomposition and nitrogen
678 mineralization from biosolids and other organic materials: relationship with initial
679 chemistry. *J. Environ. Qual.* 30: 1401-1410.

680 Ruf, A., Kuzyakov, Y., Lopatovskaya, O., 2006. Carbon fluxes in soil food webs of
681 increasing complexity revealed by ^{14}C labelling and ^{13}C natural abundance. *Soil Biol.*
682 *Biochem.* 38: 2390-2400.

683 Rumpel, C., Eusterhues, K., Kögel-Knabner, I., 2004. Location and chemical
684 composition of stabilized organic carbon in topsoil and subsoil horizons of two acid
685 forest soils. *Soil Biol. Biochem.* 36: 177-190.

686 Rumpel, C., Kögel-Knabner, I., Bruhn, F., 2002. Vertical distribution, age, and chemical
687 composition of organic carbon in two forest soils of different pedogenesis. *Org.*
688 *Geochem.* 33:1131-1142.

689 Schenk, H.J., Jackson, R.B., 2005. Mapping the global distribution of deep roots in
690 relation to climate and soil characteristics. *Geoderma* 126: 129-140.

691 Schimel, D.S., House, J.I., Hibbard, K.A., Bousquet, P., Ciais, P., Peylin, P., Braswell,
692 B.H., Apps, M.J., Baker, D., Bondeau, A., Canadell, J., Churkina, G., Cramer, W.,
693 Denning, A.S., Field, C.B., Friedlingstein, P., Goodale, C., Heimann, M., Houghton,
694 R.A., Melillo, J.M., Moore, III B., Murdiyarso, D., Noble, I., Pacala, S.W., Prentice, I.C.,
695 Raupach, M.R., Rayner, P.J., Scholes, R.J., Steffen, W.L., Wirth, C., 2001. Recent
696 patterns and mechanisms of carbon exchange by terrestrial ecosystems. *Nature* 414: 169-
697 172.

698 Shibu, M.E., Leffelaar, P.A., Van Keulen, H., Aggarwal, P.K., 2006. Quantitative
699 description of soil organic matter dynamics-A review of approaches with reference to
700 rice-based cropping systems. *Geoderma* 137: 1-18.

701 Six, J., Conant, R.T., Paul, E.A., Paustian, K., 2002. Stabilization mechanisms of soil
702 organic matter: Implications for C-saturation of soils. *Plant Soil* 241: 155-176.

703 Six, J., Frey, S.D., Thiet, K., Batten, K.M., 2006. Bacterial and fungal contributions to
704 carbon sequestration in agroecosystems. *Soil Sci. Soc. Am. J.* 70: 555-569.

705 Skjemstad, J.O., Dalal, R.C., Janik, L.J., McGowan, J.A., 2001. Changes in chemical
706 nature of soil organic carbon in Vertisols under wheat in south-eastern Queensland. *Aust.*
707 *J. Soil Res.* 39: 343-359.

708 Stankiewicz, B.A., Hutchins, J.C., Thomson, R., Briggs, D.E.G., Evershed, R.P., 1997.
709 Assessment of bog-body tissue preservation by pyrolysis-gas chromatography/mass
710 spectrometry. *Rapid. Commun. Mass Sp.* 11: 1884-1890.

711 Stevenson, F., 1994. *Humus chemistry: genesis, composition, reactions.* Wiley, New
712 York.

713 Trinsoutrot, I., Jocteur Monrozier, L., Cellier, J., Waton, H., Alamercery, S., Nicolardot,
714 B., 2001. Assessment of the biochemical composition of oilseed rape (*Brassica napus* L.)
715 ¹³C-labelled residues by global methods, FTIR and ¹³C NMR CP/MAS. *Plant Soil* 234:
716 61-72.

717 Ussiri, D.A.N., Johnson, C.E., 2003. Characterization of organic matter in a northern
718 hardwood forest soil by ¹³C NMR spectroscopy and chemical methods. *Geoderma* 111:
719 123-149.

720 van Bergen, P.F., Blokker, P., Collinson, M.E., Sinninghe Damsté, J.S., de Leeuw, J.W.,
721 2004. Structural biomacromolecules in plants: what can be learnt from the fossil record?
722 In: A.R. Hemsley and I. Poole (Editors), *The Evolution of Plant Physiology – From*
723 *whole plants to ecosystems.* Elsevier Academic Press, London, pp. 133-154.

724 van den Vossenberg, J.L.C.M., Driessen, A.J.M., Konings, W.N., 1998. The essence of
725 being extremophilic: the role of the unique archaeal membrane lipids. *Extremophiles* 2:
726 163-170.

727 Wang, W.J., Baldock, J.A., Dalal, R.C., Moody, P.W., 2004. Decomposition dynamics of
728 plant materials in relation to nitrogen availability and biochemistry determined by NMR
729 and wet-chemical analysis. *Soil Biol. Biochem.* 36: 2045-2058.

730 Weijers, J.W.H., Schouten, S., Spaargaren, O.C., Sinninghe Damsté, J.S., 2006.
731 Occurrence and distribution of tetraether membrane lipids in soils: Implications for the use
732 of the TEX₈₆ proxy and the BIT index. *Org. Geochem.* 37: 1680-1693.

733 Wiesenberg, L.B.G., Schwarzbauer, J., Schmidt, M.W.I., Schwark, L., 2004. Source and
734 turnover of organic matter in agricultural soils derived from *n*-alkane/*n*-carboxylic acid
735 compositions and C-isotope signatures. *Org. Geochem.* 35: 1371-1393.

736 Winkler, A., Haumaier, L., Zech, W., 2005. Insoluble alkyl carbon components in soils
737 derive mainly from cutin and suberin. *Org. Geochem.* 36: 519-529.

738 Wolters, V., 2000. Invertebrate control of soil organic matter stability. *Biol. Fert. Soils*
739 31: 1-19.

740 Wutzler, T., Reichstein, M., 2007. Soils apart from equilibrium - consequences for soil
741 carbon balance modelling. *Biogeosciences* 4: 125-136.

742 Yan, B., Stark, R.E., 2000. Biosynthesis, molecular structure, and domain architecture of
743 potato suberin: A ¹³C NMR study using isotopically labeled precursors. *J. Agric. Food*
744 *Chem.* 48: 3298-3304.

745 Zech, W., Guggenberger, G., 1996. Organic matter dynamics in forest soils of temperate
746 and tropical ecosystems. In: A. Piccolo (Editor), Humic Substances in Terrestrial
747 Ecosystems. Elsevier, Amsterdam, pp. 101-170.
748

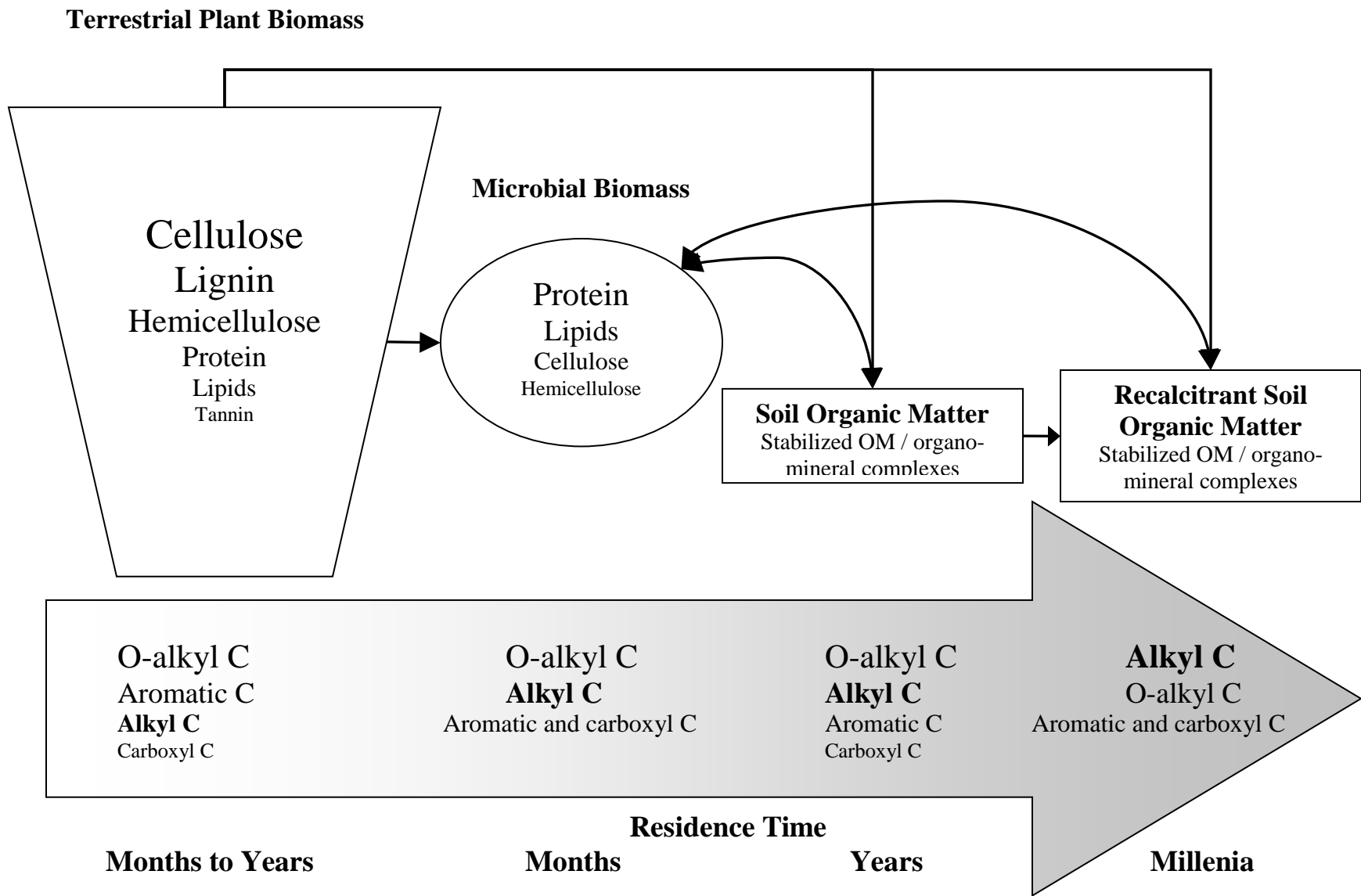


Fig. 1. C-flux through wet chemically identifiable compounds (small arrows), and relative enrichment of alkyl C (visible by NMR) (large arrow) during biodegradation of terrestrial biomass, and approximate residence times of C in plant, microbial and soil pools. Relative proportions of biomacromolecules/C-structures are indicated by different font sizes. Molecular composition and residence times are cited from Mahieu et al. (1999), Kögel-Knabner (2002), and Mikutta et al. (2006).

Table 1

Potentially recalcitrant bio(macro)molecules in terrestrial plants, microorganisms and animals (Killops and Killops, 2005; Madigan and Martinko, 2006; Rillig et al., 2007)

Chemical class	Occurrence	Bio(macro)molecule
Carbohydrates	Plant cell wall	Cellulose, hemicellulose, pectin
	Fungal cell wall	Chitin
	Bacterial cell wall	Murein or peptidoglycan, lipopolysaccharides;
	Arthropod exoskeleton, molluscs radula	Chitin
Amino acids/polypeptides/proteins	Ubiquitous in plants, microorganisms, and animals in structural and catalytic proteins	Glycoproteins, peptidoglycan, glomalin, hydrophobins, chaplins, sclerotin, collagen, fibrinogen, keratin, enzymes
Lipids: Glycerides	Plant cell (chloroplast) membrane	Phospholipids, glycolipids
	Bacterial cell membrane	Glycolipids, ether lipids
	Energy storage in animals, cell integrity	Triglycerides, phospholipids
Lipids: Waxes	Plant cuticula	Wax esters

	Plant tissue	Cutin, suberin; cutan, suberan
	Fungal cell walls	Hydrocarbons
	Bacterial cell wall	High-molecular-weight waxy molecules
	Animal coatings	Wax esters
Lipids: Terpenoids	Protective agents in plants	Di-, tri-, tetra-, and polyterpenoids; (tetrapyrroles?)
	Plant cell membrane	Steroids
	Fungal antibiotics in plants	Sesquiterpenoids
	Bacteria	Sesqui-, di-, tri-, tetra-, and polyterpenoids; steroids; tetrapyrroles
	Fungi	Tri-, tetra-, and polyterpenoids; steroids
	Protective coating of plant pollen and fungal spores (sporopollenin)	Tetraterpenoids
	Animal cell membranes and lipoproteins	Steroids, polyterpenoids
	Vitamin-A production in animals	Tetraterpenoids
Lipids: Tetrapyrrole	Primary and accessory photosynthetic,	Chlorophylls, cytochromes, phytochrome

pigments	and regulatory pigments in plants	
	Primary and accessory photosynthetic pigments in bacteria	Chlorophylls, bacteriochlorophylls, cytochromes, phycobilins
	Oxygen transport and electron transfer in animals	Haem, cytochromes
Phenolics	Plant cell walls	Lignin
	Protective agents in plants	Tannins; flavonoids; anthraquinones
	Fungi	Styrylpyrones; anthraquinones

Table 2

Turnover of plant and microbial bio(macro)molecules/fractions based on $\delta^{13}\text{C}$ analyses

Chemical class	Possible origin	Bio(macro)molecule/fraction	Residence time (yr)	References
Proteins, carbohydrates	Plants, bacteria, fungi	Proteins/amino acids/chitin	21-101	Gleixner et al. (2002)
Carbohydrates	Plants, bacteria, fungi	Cellulose, hemicellulose, pectins, chitin, peptidoglycan, lipopolysaccharides	22-125	Gleixner et al. (2002)
Lipids	Bacteria, fungi	Amino sugars	6-90	Glaser et al. (2006)
Lipids: Glycerides	Plants	<i>n</i> -Alkanes, <i>n</i> -carboxylic acids	21-35	Wiesenberg et al. (2004)
Lipids: Glycerides	Bacteria, fungi, protozoa	Phospholipid fatty acids	13-50	Burke et al. (2003)
Phenolics	Plants	Lignin	21-101	Gleixner et al. (2002)
		Lignin	0.5, 17, and 19 (litter, soil, and total)	Dignac et al. (2005), Rasse et al. (2006)

Table 3

Bio(macro)molecules/C fractions in plant biomass

Species		Cutin	Suberin	Carboxyl	Aromatic	O-alkyl	Alkyl
		----(mg m ⁻²)----		-----(% O.C.)-----			
<i>Fagus sylvatica</i> L.	Leaves	629		5	16	54	25
	Wood			2	13	83	2
<i>Picea abies</i> (L.) Karst.	Needles	833-859		6	17	60	19
	Wood			5	19	62	14
	Coarse Roots		593	4	20	69	6
	Fine roots			6	20	63	11
<i>Pseudotsuga menziesii</i>	Needles			7	18	61	25
<i>Zea mays</i> L.	Leaves			3	11	77	9
	Roots			3	9	78	10
<i>Triticum aestivum</i> L.	Roots			6	18	69	8
	Straw			5	13	71	11
<i>Brassica napus</i> L.	Stems			6	5	76	13
	Roots			5	8	75	12

References: Matzke and Riederer (1991), Gregorich et al. (1996), Rowell et al. (2001), Trinsoutrot et al. (2001), Kögel-Knabner (2002), Rosenberg et al. (2003), Lorenz et al. (2004), Wang et al. (2004), Helfrich et al. (2006).