

University of Warwick institutional repository

This paper is made available online in accordance with publisher policies. Please scroll down to view the document itself. Please refer to the repository record for this item and our policy information available from the repository home page for further information.

To see the final version of this paper please visit the publisher's website. Access to the published version may require a subscription.

Authors:	Deirdre C. Rooney, Ken Killham, Gary D. Bending, Elizabeth Baggs, Martin Weih and Angela Hodge
Title:	Mycorrhizas and biomass crops: opportunities for future sustainable development
Year of publication:	2009
Link to published version:	http://dx.doi.org/10.1016/j.tplants.2009.08.004
Publisher statement:	None

1 **Mycorrhizas and biomass crops: opportunities for future sustainable**
2 **development**

3

4

5 Deirdre C. Rooney¹, Ken Killham², Gary D. Bending³, Elizabeth Baggs², Martin
6 Weih⁴ and Angela Hodge¹

7

8 ¹Department of Biology, University of York, Area 14, PO Box 373, York, YO10
9 5YW, UK

10 ²Institute of Biological and Environmental Sciences, Cruickshank Building, St.
11 Machar Drive, Aberdeen, AB24 3UU, UK

12 ³Warwick HRI, University of Warwick, Wellesbourne, Warwick, CV35 9EF, UK

13 ⁴Department of Crop Production Ecology, Box 7043, 750 07, Uppsala, Sweden

14

15 *Corresponding author:* Rooney, D.C. (deirdre.rooney@askham-bryan.ac.uk)

16

17

18

19

20

21

22

23

24

25

26 **Abstract**

27 Central to soil health and plant productivity in natural ecosystems are *in situ*
28 soil microbial communities, of which mycorrhizal fungi are an integral component,
29 regulating nutrient transfer between plants and the surrounding soil via extensive
30 mycelial networks. Such networks are supported by plant-derived carbon and are
31 likely to be enhanced under coppiced biomass plantations, a forestry practise that
32 has been highlighted recently as a viable means of providing an alternative source
33 of energy to fossil fuels, with potentially favourable consequences for carbon
34 mitigation. Here, we explore ways in which biomass forestry, in conjunction with
35 mycorrhizal fungi, can offer a more holistic approach to addressing several topical
36 environmental issues, including 'carbon-neutral' energy, ecologically sustainable
37 land management and CO₂ sequestration.

38

39 **Sustainable biomass production for future energy needs**

40 Current developments in agriculture have involved growing bioenergy crops
41 on agricultural land, with a shift in focus from yield- and quality-related issues
42 towards more sustainable forms of agriculture [1]. A major challenge facing global
43 bioenergy production is striking the balance between long-term sustainability, and
44 reaching short-term productivity goals if bioenergy is to become a viable means of
45 reducing fossil fuel dependency. 'Sustainability' in agriculture is difficult to define
46 unequivocally, but often involves minimal chemical inputs, efficient nutrient
47 recycling and enhancement of important microbial-driven processes such as
48 nutrient acquisition, decomposition and protection against pathogens. Perennial
49 crops, such as *Salix* (willow) and *Populus* (poplar) species, grown in short rotation
50 coppice (SRC) biomass plantations represent an interesting opportunity to promote

51 agricultural sustainability, by enhancing regulation of important ecological
52 processes [1]. Although willow SRC is commercially grown in several countries
53 including Sweden (~14000 ha), the production of biomass for energy from SRC
54 forestry is still in the experimental stages of development in major countries such
55 as the UK and US [2]. Land availability and biomass yield are major concerns
56 surrounding biomass as an energy source [3-7], yet the contribution of biomass
57 derived energy to global renewable energy stocks (~50% in 2004) remains
58 significant [8].

59 Less attention, however, has focussed on sustainable SRC culture,
60 including interactions with soil microbial communities. As primary producers, plants
61 provide photosynthetically derived carbon to the soil microbial community,
62 including symbiotic mutualists, decomposers and pathogens [9] via rhizodeposition
63 [10] which is easily assimilated by the soil microbial biomass [11,12]. A vital
64 component of the soil microbial community, mycorrhizal fungi, represent the
65 primary interface between photosynthate and soil through intimate associations
66 with plant roots, and have a central role in plant nutrient acquisition and plant
67 health [13]. Interestingly, the perennial nature of SRC crops is associated with
68 minimal mechanical disturbance of soil and is likely to promote belowground
69 mycorrhizal functioning which, in turn, could promote biomass yield and cropping
70 security. Compared with conventional cropping systems, SRC plantations are only
71 replanted every 10-25 years (depending on national regulation, market issues and
72 plantation health), which significantly lengthens crop-rotation periods and the crop
73 is generally more deeply-rooted, requiring no annual soil cultivation or herbicide
74 application once established (Box 1). In addition, considerably less agrochemical
75 inputs, in particular nitrogen, are required in SRC. Non-intensive management of

76 SRC plantations may also present significant potential for belowground carbon
77 sequestration [14,15], which can improve soil quality in SRC (Box 2). Carbon
78 sequestration is especially relevant to renewable energy production as it
79 represents an additional carbon offset compared with fossil fuel emissions.

80 Understanding plant-microbial interactions is crucial to our understanding of
81 soil ecosystem function and its role in sustainable land management. Here we
82 discuss the potential importance of interactions between mycorrhizas and biomass
83 crops in SRC systems, with particular reference to the sustainable generation of
84 biomass for renewable energy and the potential for carbon sequestration.

85

86 **Mycorrhizas in biomass crop plantations**

87 Mycorrhizal fungi are an important integral component of the plant-soil
88 system, forming symbiotic associations with most land plants and mediating a
89 range of crucial ecosystem processes [13,16]. In return for photosynthetically
90 derived carbon, mycorrhizal fungi have a fundamental role in plant nutrition, most
91 notably in the provision of phosphorus and nitrogen to the host plant [13]. In
92 addition, other non-nutritional benefits, such as soil aggregation and stability [17],
93 increased drought tolerance, and protection against pathogens [18, 19] can be
94 conferred upon the associated host. Although mycorrhizas have been found
95 associated with several biomass-producing plant species, such as *Populus* and
96 *Salix* species [20-25], information concerning their functional role in SRC
97 plantations is scarce.

98 Two major types of mycorrhizal fungi, the arbuscular mycorrhizal fungi
99 (AMF) and the ectomycorrhizal fungi (ECMF), form symbiotic associations with
100 most land plants. AMF (~200 species described) are likely to have coevolved with

101 terrestrial plants, at least partially facilitating the colonisation of land by plants over
102 400 million years ago [26] and can form associations with approximately two-thirds
103 of land plants. ECMF (~6000 species described) are likely to have evolved later
104 (130 million years ago) and are generally associated with woody plants [13]. Both
105 AMF and ECMF support extensive extraradical hyphal networks maintained by
106 plant-fixed carbon (Figure 1), which act as a conduit for nutrient exchange between
107 plant roots and the soil environment. Interestingly, the main tree genera used in
108 biomass plantations worldwide (*Salix*, *Populus* and *Eucalyptus*) can form both AMF
109 and ECMF associations, occasionally with AMF and ECMF present in the same
110 root system [27]. Such 'dual-colonisation' can result from several factors, including
111 successional stages in tree development (as the tree matures and the root
112 develops) [28], availability of mycorrhizal inoculum strength (availability of fungal
113 spores and/or hyphal fragments) [25], local soil conditions [13] or geographical
114 location [29]. It is possible that different mycorrhizal types or species associated
115 with a given plant could offer functional complementarity (see Glossary) [30].
116 However, although dually-colonised trees in SRC plantations have been reported
117 [23,25,27,31], it is usually one type of mycorrhizal association that dominates or
118 exclusively colonises a given plant at any one time. Studies of willow plantations
119 revealed ECMF as the dominant mycorrhizal association, with AMF accounting for
120 significantly lower root colonisation, often <1% [20,23-25,31]. Similarly, ECMF,
121 rather than AMF, dominated both willow and poplar stands on afforested sites in
122 Northern Germany [32].

123 Growth and maintenance of mycorrhizal structures is supported by plant-
124 fixed carbon, and establishment of mycorrhizal symbioses therefore comes at a
125 carbon 'cost' to the plant. Extraradical (outside the root) hyphal growth is often

126 extensive and can account for up to 30% of the microbial biomass in soil [33,34].
127 Plant investment in mycorrhizas is offset by the benefits gained (such as increased
128 nutrient acquisition) in most cases, hence mycorrhizal associations are considered
129 mutualistic. In fact, plant investment in mycorrhizal hyphae 'cost' the plant 100
130 times less carbon to construct a unit of hypha than it would that of a root [35],
131 effectively making the symbiosis cost-effective in terms of plant carbon investment,
132 especially as hyphae can extend beyond the nutrient depletion zone that develops
133 around the roots. Plant investment in mycorrhizas should therefore decline as soil
134 nutrient availability increases, as an abundance of labile nutrients (such as fertiliser
135 additions) reduces the need for a foraging symbiont. However, the sparse
136 experimental evidence for nutrient fertilization effects on mycorrhizal colonization
137 of SRC crops indicates variable relationships: fertilization either reduced or
138 increased mycorrhizal colonisation of SRC willow, depending on soil and/or other
139 site-specific conditions [20]. Increased understanding about the relationships
140 between soil nutrient availability and mycorrhizal colonisation in SRC could be
141 used to develop marginal land for SRC forestry, thus reducing competition for high-
142 quality agricultural land, which is increasingly in demand to fulfil global food and
143 housing requirements [36].

144

145 **The role of mycorrhizas in soil carbon cycling**

146 A main feature of mycorrhizal symbioses is carbon flux from the plant to the
147 fungal symbiont, making mycorrhizas an integral link in global carbon cycling.
148 Mycorrhizal colonisation alters the carbon metabolism of the plant, increasing the
149 carbon allocation to the whole root system [37], with a significant proportion (4-
150 20%) diverted to the fungal component [12,38,39]. ¹³CO₂ labelling experiments

151 have indicated that carbon translocation to fungal hyphae can be rapid (within 24
152 hr of fixation; [38]), although slower delivery rates of up to 4 days post-labelling
153 have also been reported [40]. Poplar and willow species support substantial root
154 systems, presumably with a significant proportion of fixed carbon allocated to the
155 roots, which can be stored in the root system to support new shoot development
156 following a coppicing cycle [41]. It is currently unknown how mycorrhizal carbon
157 flux is altered during coppicing cycles, especially with regards to carbon allocation
158 to the fungal component when the aboveground biomass is harvested. It is
159 possible that coppicing causes the plant to initially retain its carbon store for self-
160 regeneration, until there is 'need' to divert some to the fungal component (i.e. when
161 mineral nutrients become limiting for growth).

162 Characteristic mycorrhizal exudates including amino acids, organic acids,
163 sugars and polysaccharides have been identified [42-45] and can be quickly
164 assimilated by the soil microbial biomass. Additionally, other fungal-specific
165 exudates, such as glomalin (a fungal glycoprotein), are produced by AMF.
166 Glomalin is highly persistent in soil (residence time of 4-62 yr) and acts as soil
167 'glue', which can improve soil structure by enhanced soil aggregation [46,47].
168 Qualitative and quantitative differences in mycorrhizal exudates might also
169 contribute to soil chemical, physical and biological heterogeneity, creating hotspots
170 of microbial activity and promoting soil activity. However, incorporation of recently
171 fixed carbon into the soil microbial biomass represents only one route for the total
172 diverted carbon, with a substantial carbon diversion to other fungal structures,
173 particularly investment in the external mycelial network. Carbon turnover from fine
174 AMF hyphae can be rapid (5-6 days) with thicker hyphae taking up to 30 days [48],
175 thus representing an important pathway by which plant-assimilated carbon enters

176 the soil environment [49]. This is in contrast to root turnover, which can take
177 several years depending on the root diameter and plant species [50]. Difficulties
178 arise in attempting to apply these turnover times to all fungal tissues, however,
179 especially if considering the carbon investment associated with the extraradical
180 mycelial network or characteristic fungal structures. Intraradical vesicles,
181 reproductive spores, arbuscules (in AMF), intra- and extraradical hyphae
182 collectively consume a large fraction of carbon allocated to the fungus. This carbon
183 pool is likely to have a much longer mean residence time in soil [51] than 5-6 days;
184 an observation which is supported by data suggesting that the residence time of
185 carbon in ECMF communities is 4-5 years [52]. Collectively, these data suggest
186 that mycorrhizas contribute to short and long-term soil organic carbon pools [47,
187 51].

188 In terms of carbon sequestration, long-term belowground storage of plant-
189 fixed carbon in stable organic forms derived from fungal spores and glomalin (the
190 latter by AMF only) offers a means of carbon storage in a relatively stable form.
191 Soil organic matter accumulation was shown to significantly increase in both willow
192 and poplar biomass plantations in the six years following afforestation of arable
193 sites, which was attributed to inputs from leaf and root litter from the newly
194 established stands [32]. Additionally, this increase in organic matter content was
195 implicated in the increased ECMF associations in the same willow and poplar
196 plantations [32]. An interesting concept arising from biomass production is that of
197 biochar generation. Biochar is a derivative of biomass carbon, formed when
198 biomass is partially combusted (in the absence of oxygen) to generate energy.
199 Such partial combustion typically releases ~50% of the carbon contained in the
200 biomass and produces a carbon-rich powdery substance (biochar) as an end

201 product, which can be added back to the soil for long term storage. Biochar has the
202 potential to sequester up to 40% of initial biomass carbon owing to its long
203 residence time in soil (thousands of years) compared with complete combustion,
204 which retains ~3% carbon, and decomposition, which can sequester only up to
205 20% carbon after ten years [53]. Biochar addition to soil can also have positive
206 effects on mycorrhizal status, notably with increases in root colonisation [54]. The
207 effects of biochar on mycorrhizas can be attributed to changes in soil physico-
208 chemical factors, such as nutrient availability and microbial activity. The exact
209 mechanisms governing mycorrhizal responses to biochar in soil require further
210 investigation however, particularly with regards to ERM dynamics. Quantification of
211 the contributions of biochar to soil carbon storage in SRC systems is also an
212 important consideration for future energy production from biomass (Box 2).

213

214 **SRC, nutrient cycling and mycorrhizas**

215 Coppicing is practiced in forestry as a means of removing apical dominance
216 to encourage accelerated growth and increased yields, and in SRC plantations
217 coppicing typically occurs every 3-5 years [41]. Following coppicing, re-growth of
218 new plant biomass is facilitated by the regeneration of new shoots from the
219 remaining stump. Conventional land management practices often include tillage
220 and significant inputs of nutrients, herbicides and pesticides can have negative
221 impacts on the number of mycorrhizal species present and can, in effect,
222 marginalise mycorrhizal and microbial functioning [19,55,56]. In commercial SRC
223 culture, the use of herbicides is required only during establishment of the plantation
224 and pesticide application is generally not required [57]. SRC plantations could
225 therefore be managed organically after the establishment phase, as a total

226 absence of fertilisation could allow biomass yield to decline to economically
227 unfeasible levels. Extrapolating these data to SRC biomass plantations is difficult
228 however, as interactions with other site-specific variables can alter mycorrhizal
229 dynamics in soil.

230 Preservation of soil microbiology in less intensively managed sites can
231 contribute to self-regulation of fundamental ecosystem processes, particularly
232 nutrient recycling, without need for further nutrient inputs. Given the support for
233 mycorrhizal enhancement of plant nutrient status [13], however, the problem of
234 nutrient limitation of yield can be alleviated by mycorrhizal retrieval of nitrogen and
235 phosphorus from soil organic material. High-yielding perennial trees, such as those
236 grown in biomass plantations, can generally produce high dry matter yields from
237 modest nitrogen applications (20-50% less nitrogen fertilisation than annual crops)
238 [41]. This may mean that nitrogen inputs to SRC plantations can often be kept to a
239 minimum (at least compared to many conventional agricultural practices), thereby
240 minimising the possibility of adverse environmental impacts which may ensue if
241 excess nitrogen is applied to the land. Closure of major nutrients cycles, such as
242 nitrogen cycling, is one of the most important factors in ecologically sustainable
243 systems, as it lowers the amount of nitrogen leached out or lost in gaseous form.

244 Enhancing the availability of phosphorus and nitrogen to host plants is
245 considered the most important function of mycorrhizas [13], and nutrient availability
246 in sustainable systems is often dependent on mycorrhizal activity. Phosphorus is a
247 major nutrient required by plants, although in soil it is usually present in very low
248 concentrations [13], as soluble phosphorus is readily taken up by both plants and
249 microbes. Investment in mycorrhizas therefore means that plants can indirectly
250 access nutrients beyond the nutrient depletion zone of the roots via extensive

251 mycelial networks. Both AMF and ECMF can effectively forage for relatively
252 insoluble forms of soil inorganic phosphorus, such as rock phosphate, iron
253 phosphates and aluminium phosphates [13]. In addition, ECMF have a major role
254 in recycling soil organic nitrogen and organic phosphorus which are unavailable to
255 the plant. In some forests, ECMF can suppress the activity of saprotrophs, a
256 process known as 'the Gadgil effect', whereby ECMF inhibition of saprotrophic
257 microbes was implicated in reduced litter decomposition, allowing accumulation of
258 organic matter in the soil [58]. This could have been due to ECMF being supplied
259 with energy from their plant host, which could give them a competitive advantage
260 over saprotrophs. Although it is not clear how these processes are regulated in
261 SRC forests, these actions suggest a possible niche role for efficient nutrient
262 cycling under low-input systems. By contrast, the role of AMF in retrieval of organic
263 nitrogen is unclear as AMF are not known to have any saprotrophic capabilities,
264 although AMF involvement in nitrogen capture from complex organic sources has
265 previously been demonstrated in laboratory conditions [59,60].

266

267 **Effects of mycorrhizas on soil biodiversity**

268 Mycorrhizal persistence under sustainably managed SRC could promote
269 soil biological diversity through further symbiotic interactions with important soil
270 organisms. For example, specific bacterial groups often associate with mycorrhizal
271 hyphae [61], including plant growth-promoting rhizobacteria (PGPR) [62,63] which
272 are important contributors to overall plant growth and/or nutrition. In addition, some
273 bacterial communities were shown to specifically attach to dead AMF hyphae,
274 whereas others used exudates from living hyphae as a growth substrate, the latter
275 including two known PGPRs (*Pseudomonas fluorescens* SBW25 and *Paenibacillus*

276 *brailensis* PB177) [64]. Mycorrhiza helper bacteria (MHB) have been identified as
277 important components of both AMF and ECMF hyphospheres and are capable of
278 increasing rates of mycorrhizal colonisation and suppressing soil pathogens [65]. A
279 recent study demonstrated the effectiveness of introducing both selected
280 mycorrhizal fungal and bacterial species to poplar seedlings at the nursery stage,
281 where co-inoculation increased plant nutrient status and increased establishment
282 success at various sites [66]. Furthermore, interactions with higher trophic
283 organisms are enhanced by mycorrhizal presence, in particular hyphal predators
284 such as collembolans, nematodes and mites [67,68], although little is currently
285 known about the underlying mechanisms that govern these interactions. Hyphal
286 grazing emphasises a further positive impact that mycorrhizas can have on soil
287 food webs and soil biodiversity, the consequences of which might mean greater
288 ecosystem productivity [69] and greater soil carbon storage. Difficulties are faced
289 when attempting to extrapolate this sparse knowledge, derived mainly from
290 microcosm work, to the field and specifically to SRC biomass plantations.

291 Another interesting example regarding the effects of mycorrhizas on higher
292 trophic organisms is represented by the interactions between root mycorrhizal
293 colonization and leaf herbivore resistance in willows [70]. In fact, mycorrhizas and
294 their influence on crop resistance to phytophagous insects might involve a yet
295 unexplored potential for the bioprotection of agricultural crops. Control of pests and
296 diseases in biomass plantations is important for the maintenance of high yields (i.e.
297 crop security). Plant resistance to insect attack is often mediated by tissue
298 concentrations of phenolic compounds that affect insect behaviour, development
299 and survival. In an experimental study, the effects of mycorrhizas on willow leaf
300 chemistry were found to be dependent on specific combinations of fungal species

301 and plant genotype [70]. Mycorrhizal control over the production of plant foliar
302 substances (e.g. salicylic acid) could therefore present opportunities for selection
303 of compatible fungal and plant combinations to combat herbivory. In effect, this
304 presents a conceptual basis for the development of biological control strategies
305 against insect herbivory in willow and poplar biomass plantations.

306

307 **Challenges for future research**

308 The theoretical potential of bioenergy is vast, and considerable emphasis
309 must be placed on conducting large-scale field trials to optimise biogeochemical
310 conditions for sustainable biomass production. Many groups of organisms regulate
311 essential ecosystem processes, but mycorrhizal fungi arguably represent the most
312 important keystone group linking crop productivity and cropping security to below
313 ground functioning (Figure 2). The preservation of mycorrhizal status in biomass
314 plantations could significantly improve the viability of low-input SRC plantations.
315 However, lack of information regarding the underlying functional relationships
316 between plants, mycorrhizas, pests and microorganisms represents a major
317 challenge in the attempt to achieve sustainability. We have identified the following
318 areas which merit further investigation: the effects of mycorrhizas on biomass
319 production and carbon sequestration under various management regimes; the
320 effects of nutrient fertilization, regular harvests and biochar application on
321 mycorrhizal functioning; the interactions between mycorrhizal fungal genotype and
322 crop genotype. Another prerequisite for the successful implementation of
323 sustainable management concepts into SRC culture is the consideration of
324 ecological processes in crop breeding, as specific fungal – host genotype
325 combinations seem to be crucial for the ultimate effects of mycorrhizas on crop

326 performance and pest resistance [70,71]. Resolution of such issues will contribute
327 greatly to our understanding of how sustainable land management and future
328 energy needs may be achieved.

329 Depletion of finite resources, such as global phosphate reserves, which are
330 estimated to run out ~100-150 years from now, based on current exploitation rates
331 [77, 78] suggests that management of organisms, such as mycorrhizal fungi, which
332 can exploit and recycle soil phosphorus and nitrogen is advantageous. Integration
333 of mycorrhizal systems with other carbon sequestration management practices, for
334 example biochar usage [53,54] (Box 2), could also be an important future practice.
335 The first mycorrhizal symbioses evolved over 400 million years ago in response to
336 phosphorus deficiency in terrestrial ecosystems [35], and we suggest that
337 capitalisation on this strategy in sustainably managed ecosystems could become
338 essential for future land management and crop production.

339

340 **Acknowledgements**

341 Funding for this work was supported by the Biotechnology and Biological Sciences
342 Research Council.

343

344 **References**

345 1 Jordan, N. *et al.* (2007) Sustainable development of the agricultural bio-
346 economy. *Science* 316, 1570-1571

347 2 Kuzovkina, Y. *et al.* (2008) *Salix*: Botany and Global Horticulture. *Horticultural*
348 *Reviews* 34, 447-489

349 3 Klass D.L. (1998) *Biomass for Renewable Energy, Fuels and Chemicals* Elsevier

- 350 4 Grogan, P. and Matthews, R. (2002) A modelling analysis of the potential for soil
351 carbon sequestration under short rotation coppice willow bioenergy plantations.
352 *Soil Use Manage.* 18, 175-183
- 353 5 Fang, S. *et al.* (2007) Biomass production and carbon sequestration in poplar
354 plantations with different management patterns. *J. Environ. Manage.* 85, 672-679
- 355 6 Dillen, S.Y. *et al.* (2007) Effects of environment and progeny on biomass
356 estimations of five hybrid poplar families grown at three contrasting sites across
357 Europe. *For. Ecol. Manage.* 252, 12-23
- 358 7 Aylott, M.J. *et al.* (2008) Yield and spatial supply of bioenergy poplar and willow
359 short-rotation coppice in the UK. *New Phytol.* 178, 358-370
- 360 8 Sims, R.E.H. *et al.* (2007) Energy supply. In *Climate Change 2007: Mitigation.*
361 *Contribution of the Working Group III to the Fourth Assessment Report of the*
362 *International Panel on Climate Change* (Metz B. *et al.*, eds), pp. 252-322
363 Cambridge University Press
- 364 9 Wardle, D.A. *et al.* (2004) Ecological linkages between aboveground and
365 belowground biota. *Science* 304, 1629-1633
- 366 10 Jones, D.L. *et al.* (2004) Plant and mycorrhizal regulation of rhizodeposition.
367 *New Phytol.* 163, 459-480
- 368 11 Paterson, E. *et al.* (2006) Rhizodeposition shapes rhizosphere microbial
369 community structure in organic soil. *New Phytol.* 173, 600-610
- 370 12 Högberg, P. *et al.* (2001) Large-scale forest girdling shows that current
371 photosynthesis drives soil respiration. *Nature* 411, 789-792

- 372 13 Smith, S.E. and Read, D.J. (2008) *Mycorrhizal Symbiosis* (3rd edn) Academic
373 Press
- 374 14 Liebig, M.A. *et al.* (2005) Soil carbon under switchgrass stands and cultivated
375 cropland. *Biomass Bioenergy* 28, 347-354
- 376 15 Lal, R. (2003) Global potential of soil carbon sequestration to mitigate the
377 greenhouse effect. *Crit. Rev. Plant Sci.* 22, 151-184
- 378 16 van der Heijden, M.G.A. *et al.* (2008) The unseen majority: soil microbes as
379 drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol. Lett.* 11,
380 296-310
- 381 17 Moreno-Espandola, I.P. *et al.* (2007) Role of root-hairs and hyphae in adhesion
382 of sand particles. *Soil Biol. Biochem.* 39, 2520-2526
- 383 18 Azcon-Aguilar, C. *et al.* (2002) The contribution of arbuscular mycorrhizal fungi
384 to the control of soil borne pathogens. In *Mycorrhizal Technology in Agriculture*
385 (Gianinazzi, S. *et al.*, eds), pp. 187-198 Springer
- 386 19 Gosling, P. *et al.* (2006) Arbuscular mycorrhizal fungi and organic farming.
387 *Agric. Ecosyst. Environ.* 113, 17-35
- 388 20 Baum, C. *et al.* (2002) The effects of nitrogen fertilisation and soil properties on
389 mycorrhizal formation of *Salix viminalis*. *Forest. Ecol. Manage.* 160, 35-43
- 390 21 Baum C. *et al.* (2002) Growth response of *Populus trichocarpa* to inoculation by
391 the ectomycorrhizal fungus *Laccaria laccata* in a pot and a field experiment. *Forest*
392 *Ecol. Manage.* 163, 1-8

- 393 22 Khasa, P.D. *et al.* (2002) The mycorrhizal status of selected poplar clones
394 introduced in Alberta. *Biomass Bioenergy* 22, 99-104
- 395 23 Hashimoto, Y. and Higuchi, R. (2003) Ectomycorrhizal and arbuscular
396 mycorrhizal colonisation of two species of floodplain willows. *Mycoscience* 44. 339-
397 343.
- 398 24 Püttsepp U. *et al.* (2004) Ectomycorrhizal fungal communities associated with
399 *Salix viminalis* L. and *S. dasyclados* Wimm. clones in a short-rotation forestry
400 plantation. *For. Ecol. Manage.* 196, 413-424
- 401 25 Trowbridge, J. and Jumpponen, A. (2004) Fungal colonisation of shrub willow
402 roots at the forefront of a receding glacier. *Mycorrhiza* 14, 283-293
- 403 26 Simon, L. *et al.* (1993) Origin and diversification of endomycorrhizal fungi and
404 coincidence with vascular land plants. *Nature* 363, 67-69
- 405 27 Chen, Y. *et al.* (2000) Effects of ectomycorrhizas and vesicular-arbuscular
406 mycorrhizas, alone or in competition, on root colonisation and growth of *Eucalyptus*
407 *globulus* and *E. urophylla*. *New Phytol.* 146, 545-556
- 408 28 van der Heijden, E. (2001) Differential benefits of arbuscular mycorrhizal and
409 ectomycorrhizal infection of *Salix repens*. *Mycorrhiza* 10, 185-193
- 410 29 Read, D.J. (1991) Mycorrhizas in ecosystems. *Experientia* 47, 376-391
- 411 30 Jansa, J. *et al.* (2007) Are there benefits of simultaneous root colonisation by
412 different arbuscular mycorrhizal fungi? *New Phytol.* 177, 779-789

- 413 31 Gehring C.A. *et al.* (2006) Environmental and genetic effects on the formation of
414 ectomycorrhizal and arbuscular mycorrhizal associations in cottonwoods.
415 *Oecologia* 149, 158-164
- 416 32 Kahle *et al.* (2005) Effects of afforestation on soil properties and mycorrhizal
417 formation. *Pedosphere* 15, 754-760
- 418 33 Leake, J.R. *et al.* (2004) Networks of power and influence: the role of
419 mycorrhizal mycelium in controlling plant communities and agroecosystem
420 functioning. *Can. J. Bot.* 82, 1016-1045
- 421 34 Högborg, M and Högborg, P. (2002) Extramatrical ectomycorrhizal mycelium
422 contributes one-third of microbial biomass and produces, together with associated
423 roots, half the dissolved organic carbon in a forest soil. *New Phytol.* 154, 791-795
- 424 35 Helgason, T. and Fitter, A. The ecology and evolution of the arbuscular
425 mycorrhizal fungi. *Mycologist* 19, 96-101
- 426 36 Lal, R. (2007) Soil science and the carbon civilisation. *Soil Sci. Soc. Am. J.* 71,
427 1425–1437
- 428 37 Jones, M.D. *et al.* (1991) Fluxes of carbon and phosphorus between symbionts
429 in willow ectomycorrhizas and their changes with time. *New Phytol.* 119, 99-106
- 430 38 Johnson, D. *et al.* (2002) In situ ¹³CO₂ pulse-labelling of upland grassland
431 demonstrates a rapid pathway of carbon flux from arbuscular mycorrhizal mycelia
432 to the soil. *New Phytol.* 153, 327-334
- 433 39 Hodge, A. (1996) Impact of elevated CO₂ on mycorrhizal associations and
434 implications for plant growth. *Biol. Fertil. Soils* 23, 388-398

435 40 Ekblad, A. and Högberg, P. (2001) Natural abundance of ^{13}C in CO_2 respired
436 from forest soils reveals speed of link between tree photosynthesis and root
437 respiration. *Oecologia* 127, 305-308

438 41 Karp A. and Shield I. (2008) Bioenergy from plants and the sustainable yield
439 challenge. *New Phytol.* 179, 15-32

440 42 Read, D.J. and Perez-Moreno, J. (2003) Mycorrhizas and nutrient cycling in
441 ecosystems- a journey towards relevance? *New Phytol.* 157, 475-492

442 43 Landeweert, R. *et al.* (2001) Linking plants to rocks: ectomycorrhizal fungi
443 mobilize nutrients from minerals. *Trends Ecol. Evol.* 16, 248-254

444 44 Hooker, J.E. *et al.* (2007) Polysaccharides and monosaccharides in the
445 hyphosphere of the arbuscular mycorrhizal fungi *Glomus* E3 and *Glomus tenue*.
446 *Soil Biol. Biochem.* 39, 680-683

447 45 Toljander, J.F. *et al.* (2007) Influence of arbuscular mycorrhizal mycelial
448 exudates on soil bacterial growth and community structure. *FEMS Microbiol. Ecol.*
449 61, 295-304

450 46 Rillig, M.C. (2004) Arbuscular mycorrhizae, glomalin and soil aggregation. *Can.*
451 *J. Soil Sci.* 84, 355-363

452 47 Zhu, Y.G. and Miller, R.M. (2003) Carbon cycling by arbuscular mycorrhizal
453 fungi in soil-plant systems. *Trends Plant Sci.* 8, 407-409

454 48 Staddon, P.L. *et al.* (2003) Rapid turnover of hyphae of mycorrhizal fungi
455 determined by AMS analysis of ^{14}C . *Science* 300, 1138-1140

456 49 Godbold, D.L. *et al.* (2006) Mycorrhizal hyphal turnover as a dominant process
457 for carbon input into soil organic matter. *Plant Soil* 281, 15-24

458 50 Matamala, R. *et al.* (2003) Impacts of fine root turnover on forest NPP and soil
459 C sequestration potential. *Science* 302, 1385-1387

460 51 Olsson, P.A. and Johnson, N.C (2005) Tracking carbon from the atmosphere to
461 the rhizosphere. *Ecol. Lett.* 8, 1264-1270

462 52 Treseder *et al.*, (2004) Species-specific measurements of ectomycorrhizal
463 turnover under N-fertilisation: combining isotopic and genetic approaches.
464 *Oecologia* 138, 419-425

465 53 Lehmann, J. *et al.* (2006) Bio-char in terrestrial ecosystems - a review. *Mitig.*
466 *Adapt. Strat. Glob. Change* 11, 403-427

467 54 Warnock, D.D. *et al.* (2007) Mycorrhizal responses to biochar in soil – concepts
468 and mechanisms. *Plant Soil* 300, 9-20

469 55 Helgason, T. *et al.* (1998) Ploughing up the wood-wide web? *Nature* 394, 431

470 56 Castillo C. *et al.* (2006) Early effects of tillage and crop rotation on arbuscular
471 mycorrhizal fungal propagules in an Ultisol. *Biol. Fertil. Soils* 43, 83-92

472 57 Larsson, S. *et al.* (2007). *Manual for SRC Willow Growers*. Lantmännen
473 Agroenergi AB (www.agroenergi.se), Örebro, Sweden. P18

474 58 Gadgil R.L and Gadgil, P.D. (1971) Mycorrhiza and litter decomposition. *Nature*
475 233, 133

476 59 Hodge, A. *et al.* (2001) An arbuscular mycorrhizal fungus accelerates
477 decomposition and acquires nitrogen directly from organic material. *Nature* 413,
478 297-301

479 60 Leigh, J. *et al.* (2009) Arbuscular mycorrhizal fungi can transfer substantial
480 amounts of nitrogen to their host plant from organic material. *New Phytol.* 181,
481 199-207

482 61 Vestergard, M. *et al.* (2008) Rhizosphere bacterial community composition
483 responds to arbuscular mycorrhiza, but not to reductions in microbial activity
484 induced by foliar cutting. *FEMS Microbiol. Ecol.* 64, 78-89

485 62 Garbaye, J. (1994) Helper bacteria: a new dimension to the mycorrhizal
486 symbiosis. *New Phytol.* 128, 197-210

487 63 Jaderlund, L. *et al.* (2008) Specific interactions between arbuscular mycorrhizal
488 fungi and plant growth-promoting bacteria: as revealed by different combinations.
489 *FEMS Microbiol. Lett.* 287, 174-180

490 64 Toljander, J.F. *et al.* (2006) Attachment of different soil bacteria to arbuscular
491 mycorrhizal fungal extraradical hyphae is determined by hyphal vitality and fungal
492 species. *FEMS Microbiol. Lett.* 254, 34-40

493 65 Bending, G.D. *et al.* (2006) Significance of microbial interactions in the
494 mycorrhizosphere. *Adv. Appl. Microbiol.* 60, 97-132

495 66 Quoreshi A.M. and Khasa D.P. (2008) Effectiveness of mycorrhizal inoculation
496 in the nursery on root colonisation, growth and nutrient uptake of aspen and
497 balsam poplar. *Biomass Bioenergy* 32, 381-391

498 67 Bardgett, R. (2005) *The biology of soil: a community and ecosystem approach*.
499 p.71, Oxford University Press

500 68 Klironomos, J.N. and Ursic, M. (1998) Density-dependent grazing on the
501 extraradical hyphal network of the arbuscular mycorrhizal fungus, *Glomus*
502 *intraradices*, by the collembolan, *Folsomia candida*. *Biol. Fertil. Soils* 26, 250-253

503 69 van der Heijden, M.G.A. *et al.* (1998) Mycorrhizal fungal diversity determines
504 plant biodiversity, ecosystem variability and productivity. *Nature* 396, 69-72

505 70 Baum C. *et al.*, (2009) The significance of host-fungus combinations in
506 ectomycorrhizal symbioses for the chemical quality of willow foliage. *Plant Soil* DOI
507 10.1007/s11104-009-9928-x

508 71 Weih, M. *et al.* (2008) Integrated agricultural research and crop breeding:
509 Allelopathic weed control in cereals and long-term productivity in perennial
510 biomass crops. *Agricultural Systems* 97: 99-107

511 72 Denison, R. and Kiers, E.T. (2005) Sustainable crop nutrition: constraints and
512 opportunities. In *Plant Nutritional Genomics* (Broadley, M.R. and White, P.J. eds),
513 pp. 242-286 Blackwell

514 73 Cohen, D. (2007) Earth's natural wealth: an audit. *New Sci.* 2605, 34-41

515 74 Tilman, J. *et al.* (2006) Carbon-negative biofuels from low-input high-diversity
516 grassland biomass. *Science* 314, 1598-1600

517 75 Field, C.B. *et al.* (2007) Biomass energy: the scale of the potential resource.
518 *Trends Ecol. Evol.* 23, 65-72

- 519 76 Fargione, J. *et al.* (2008) Land-clearing and the biofuel carbon debt. *Science*
520 319, 1235-1237
- 521 77 Searchinger, T. *et al.* (2008) Use of U.S. croplands for biofuels increases
522 greenhouse gases through emissions from land-use change. *Science* 319, 1238-
523 1240
- 524 78 Boehmel, C. *et al.* (2008) Comparing annual and perennial energy cropping
525 systems with different management intensities. *Agricultural Systems* 96: 224-236
- 526 79 Hoffmann, D. and Weih, M. (2005) Limitations and improvement of the potential
527 utilisation of woody biomass for energy derived from short rotation woody crops in
528 Sweden and Germany. *Biomass Bioenergy* 28, 267-279
- 529 80 Bostrom, B. *et al.* (2007) Isotope fractionation and ^{13}C enrichment in soil profiles
530 during the decomposition of soil organic matter. *Oecologia* 153, 89-98
- 531 81 De Schriver, A. *et al.* (2008) Nitrogen saturation and net ecosystem production.
532 *Nature* 451, 26
- 533 82 Magnani, F. *et al.* (2007) The human footprint in the carbon cycle of temperate
534 and boreal forests. *Nature* 447, 848-852

535

536 **Box 1. Biomass for bioenergy**

537 Renewable energy is currently estimated to provide only 15% of the global
538 primary energy supply [8], despite increasing concern about rises in atmospheric
539 carbon arising from fossil fuel combustion. Recent environmental commitments by
540 major countries, including the UK and USA, have focussed attention on the

541 potential of renewable bioenergy as a means of alleviating dependence on
542 depleting fossil fuel reserves and reducing CO₂ emissions. However, production of
543 biomass for energy, in particular that derived from annual crops, such as maize
544 and wheat, has been a source of much debate [74-75]. Annual crops, (crops grown
545 for one growing season), currently contribute significantly to the global bioenergy
546 market, but yields are dependent on high nutrient inputs. In contrast, perennial
547 grasses and tree crops, (crops with a life span of more than 2 yr) can achieve
548 higher biomass yields with relatively lower inputs of nitrogen fertilizer [78]. For
549 example, SRC plantations throughout Europe were capable of yielding high
550 amounts of biomass without need for fertilisers [79], suggesting that it is possible in
551 many cases to balance ecological and economic objectives by proper soil
552 management. The proportion of global energy diverted to nitrogen fertiliser
553 production and use is estimated at 2%, so fertiliser applications greatly affect the
554 overall energy cost and balance within a system.

555 Woody biomass crop production has largely been driven by the ability of
556 fast-growing members of the Salicaceae, such as *Populus* (poplar) and *Salix*
557 (willow), and other genera such as *Eucalyptus* and *Acacia* to regenerate
558 vegetatively following coppicing, with coppices typically occurring every 4-16 years,
559 although longer coppice cycles (up to 30 yr) are also practiced [3].
560 Characteristically, many poplar and willow species meet the criteria as suitable
561 species for energy harvest, including fast growth, high yield and the ability to grow
562 on marginal land. In addition, biomass plantation management could benefit from
563 the genotypic variability associated with members of the Salicaceae, whereby
564 desirable attributes, such as pest and disease resistance, are identified and
565 exploited to enhance biomass production. Practice of 'naturalistic' SRC forestry [3],

566 whereby tree species are selected based on their suitability for a particular site,
567 could have positive effects on biomass production on marginal or waste land.
568 Manipulating plant species with broad genetic variability to produce favourable
569 hybrids, plus establishing suitable combinations of plant, mycorrhizal and bacterial
570 species [70], could maximise plant growth benefits in SRC forestry. Future studies
571 should attempt to elucidate the complex interactions among the relevant
572 mycorrhizal fungi, soil microbes and commercial varieties of SRC crops in the field.

573

574 **Box 2. Carbon sequestration in SRC forestry**

575 Mitigation of global climate change can be partially achieved by increasing
576 the carbon sink of terrestrial ecosystems, most importantly through changes in land
577 use and management [15]. It is generally accepted that the potential for soil carbon
578 sequestration is enhanced under conditions of minimal disturbance, high soil
579 biomass, improved soil structure, conservative nutrient cycling, and high faunal
580 and microbial biodiversity. These factors intrinsically link soil carbon storage to
581 non-intensive land management, such as sustainably managed SRC plantations.
582 Incidentally, relatively undisturbed forests generally also have higher mycorrhizal
583 biomass (e.g. up to 30% of the microbial biomass was accounted for by ECMF in a
584 boreal forest soil [34]), which represents a significant terrestrial sink for
585 photosynthetically fixed carbon. Evidence for long-term carbon storage under SRC
586 plantations remains scarce, however, although it has been simulated that SRC
587 forests divert more carbon belowground than do regenerated woodlands [4]. In a
588 study of natural ^{13}C abundance in vertical soil gradients of a 68-yr-old Norway
589 spruce forest, older microbially derived carbon was identified as the main
590 contributor to soil respiration at soil depths below 20 cm [80]. This suggests that

591 microbial immobilisation of carbon is important in soil carbon cycling in forests.
592 Evidence of the impacts of SRC on soil carbon sequestration is lacking mainly
593 owing to problems surrounding the evaluation of slow processes such as carbon
594 sequestration on relatively recently established SRC plantations (since the 1980's)
595 [4]. Research into carbon sequestration under SRC willow and poplar in the US
596 indicated that an initial loss of soil carbon occurred during the first years after
597 plantation establishment, possibly due to enhanced decomposition. Over the 18-
598 year study, however, carbon was sequestered at an average rate of 1.6 Mg ha⁻¹yr⁻¹
599 compared with control fields, which was attributed to increases in leaf litter inputs
600 and slower rates of decomposition [4]. Effectively, biomass could be used to
601 remove surplus CO₂ from the atmosphere and, particularly when combined with
602 biochar production, offer a source of carbon neutral energy. Nitrogen addition to
603 forests has also been implicated in increased soil carbon sequestration [81]
604 although at present there is much debate on this topic, particularly with regards to
605 possible increases in greenhouse gases emissions, such as methane and nitrous
606 oxide [82]. Although development of strategies to increase future soil carbon
607 storage will require further study, land management strategies that incorporate the
608 use of biochar (see Glossary), such as sustainable SRC plantations, could have
609 particular significance for soil carbon sequestration in the long term.

610

611 **Glossary**

612 **Arbuscular mycorrhizal fungi (AMF):** members of the monophyletic group,
613 Glomeromycota, characterised by formation of distinct intracellular 'arbuscules'
614 within the root system.

615 **Biochar:** a derivative of biomass carbon, formed when biomass is partially
616 combusted in the absence of oxygen.

617 **Bioenergy:** energy that is sourced from biologically derived matter, including
618 combustible woodfuel, wood waste, crop residues, municipal waste and ethanol
619 production from cereals and other crops.

620 **Biomass energy:** carbon-based renewable energy derived from plant matter.

621 **Carbon-neutral energy:** energy consumption whereby the carbon released does
622 not increase current atmospheric carbon levels.

623 **Cropping security:** the protection of economically valuable crops and yields from
624 the effects of potential stresses including extreme climate events, pests, diseases
625 and invasive species.

626 **Ectomycorrhizal fungi (ECMF):** characterised by hyphal growth between root
627 cortical cells, known as the Hartig net, often with a fungal sheath (mantle)
628 enclosing root tips.

629 **Extraradical mycelia:** external (outside root) phase of mycorrhizal fungi, formed
630 by both AMF and ECMF hyphae, extending the area over which nutrients can be
631 taken up or released.

632 **Functional complementarity:** (in mycorrhizas) performance of different functions
633 by mycorrhizal fungal species, which confer contrasting benefits on the associated
634 plant.

635 **Mycorrhizal hyphosphere:** volume of soil influenced biologically, chemically or
636 physically by mycorrhizal fungal hyphae.

637 **Mycorrhizas:** literally 'fungus-root', a symbiotic association between plants and
638 soil fungi.

639 **Mycorrhizosphere:** volume of soil influenced biologically, chemically or physically
640 by both growing plant roots (often colonised by mycorrhizas) and mycorrhizal
641 fungal hyphae.

642 **Plant-growth promoting rhizobacteria (PGPR):** soil bacteria that positively affect
643 plant growth and/or nutrition, either directly or indirectly.

644 **Rhizodeposition:** carbon release to soil via plant roots (including the mycorrhizal
645 component), which includes passive exudation of low molecular weight
646 compounds, active secretion of high molecular weight compounds, lysates
647 released from dead root cells, mucilages and dead roots.

648 **Rhizosphere:** volume of soil influenced biologically, chemically or physically by the
649 growing plant root.

650 **Short rotation coppice (SRC):** forestry practice of removing aboveground plant
651 biomass only, allowing vegetative regeneration of the next crop. Coppicing typically
652 occurs every 3-5 years.

653

654 **Figure 1.** *Populus* (poplar) roots with ectomycorrhizal and arbuscular mycorrhizal
655 structures. (a) fine poplar root tips are covered with an ectomycorrhizal sheath (s)
656 with thread-like hyphae extending from the root creating a mycelial network (m). An
657 outer sheath-like structure or fungal mantle often encloses fine root tips that have
658 been colonised by ectomycorrhizal fungi; (b) intracellular arbuscule (arb)
659 invaginating a poplar root cell. Arbuscules are a characteristic feature of arbuscular
660 mycorrhizas and nutrient exchange probably occurs here. Scale bars: (a) 0.3mm
661 (b) 10 μm

662 **Figure 2.** Potential effects of mycorrhizas on biomass crop functions and
663 performance (yield and cropping security). Photo: Short rotation coppice plantation
664 on agricultural land near Uppsala, central Sweden (M. Weih)