Reply to Anonymous Referee #1, bdg-8-C1914-2011

For the completeness of the review, it is suggested that this section is extended with a brief general introduction to the concept of the delta unit / atom% and reference materials; in this context it is also important to stress the difference between the 'little' delta expressing 13/12 C ratios, and the 'large' delta expressing isotopic fractionation during transformations (mentioned in 2.1).

→The information has been added to the introduction according to the referee's suggestions. The section now reads as follows:
"These result in the 'preference' of chemical and physical processes for one isotopologue, usually the lighter one, over the other (e.g. preference for $^{12}CO_2$ over $^{13}CO_2$) and hence in so-called *fractionation* events, which change the isotopic composition of compounds involved in such processes. The carbon isotope composition is usually expressed in δ-notation (in ‰ units), relative to the international standard Vienna Pee Dee Belemnite (VPDB) (Hut 1987). The carbon isotopic composition $\delta^{13}C$ of any sample is thus expressed as deviation from VPDB as shown in Eqn. 1:

$$\delta^{13}C = \frac{R_{sample}}{R_{VPDB}} - 1 \qquad \textbf{Equation (1)}$$

where R is the isotope (abundance) ratio ($^{13}C/^{12}C$) of a given sample (R_{sample}) and of VPDB (R_{VPDB} = 0.0111802; from Werner and Brand, 2001), respectively.
The notation for isotope fractionation is the capital Greek letter Δ. Carbon isotope discrimination ($\Delta^{13}C$) is defined as the depletion of ^{13}C during any process preferring the lighter isotopologue:

$$\Delta^{13}C = \frac{\delta^{13}C_s - \delta^{13}C_p}{1 + \delta^{13}C_p} \qquad \textbf{Equation (2)}$$

where $\delta^{13}C_s$ is the carbon isotope signature of the source (or the substrate entering a reaction; e.g. CO_2 when photosynthetic fractionation is considered) and $\delta^{13}C_p$ is the isotopic signature of the product of a process (Farquhar et al., 1982)."

The section 2 deals with carbon fractionation in plants. It is suggested that this section is elaborated a bit further to include an overview of the discrepancies among different photosynthetic systems (C3-C4-CAM), which has different impacts of the 13C of assimilated C.

→ According to the referee's suggestions we have added a paragraph on photosynthetic discrimination associated with C4 and CAM photosynthesis. The section reads as follows:
"Carbon isotope discrimination related to C_4 photosynthesis is much smaller and less variable compared to the C_3 pathway. Net fractionation of the CO_2 fixation by the enzyme phosphoenolpyruvate carboxylase (PEPc) in the mesophyll cell is -5.7‰, i.e. there is a discrimination against the lighter carbon, ^{12}C (Farquhar, 1983). This is mainly due to the fact that PEPc uses HCO_3^- as substrate and the dissolution plus hydration of CO_2 enriches ^{13}C in HCO_3^- by 7.9‰ (at 25°C; Mook et al., 1974) , and PEPc discriminates by only 2.2‰ against ^{13}C. The PEPc-fixed CO_2 will be released again in the bundle sheath cells, where it is re-fixed by Rubisco. Since part of the CO_2 released in the bundle sheath tissue leaks out to the mesophyll (Hatch, 1996), a (metabolic) branching point is formed, which allows ^{13}C discrimination by

Rubisco (Farquhar, 1983). Farquhar (1983) developed the following (simplified) equation to describe the carbon isotope discrimination of C_4 photosynthesis:

$$\Delta = a + (b_4 + b_3\phi - a)\frac{p_i}{p_a} \qquad \textbf{Equation (6)}$$

where b_4 describes the discrimination of the fixation of gaseous CO_2 in equilibrium with HCO_3^- (at 25°C) by PEPc (for details see Farquhar, 1983 and Farquhar et al., 1989), ϕ is the relative proportion of the carbon fixed by PEPc that leaks out of the bundle sheath ("bundle sheath leakiness"; Farquhar, 1983) and b_3 describes the discrimination by Rubisco.
The Crassulacean Acid metabolism (CAM) as a particular modification of the photosynthetic carbon fixation also imprints a specific carbon isotope signal on the assimilates (O'Leary, 1988). The most simple description of the CAM according to Lüttge (2004) is that there is nocturnal uptake of CO_2 via open stomata, CO_2 fixation by PEPc and vacuolar storage of organic acid assimilates, mainly malic acid (phase I; Osmond, 1978), and daytime remobilization of vacuolar organic acids, decarboxylation and re-fixation of the released CO_2 behind closed stomata by Rubisco (phase III). The malate stored at night will show the same discrimination as for C_4 species without bundle sheath leakiness and since CO_2 evolution during phase III is assumed to be negligible, the carbon isotope discrimination in phase I and III might be described by eqn. 6 assuming ϕ to be 0 (Farquhar et al. 1989). In the early light period (phase II) and in the late light period, when organic acids are exhausted (phase VI), however, stomata are open and external CO_2 can be fixed by Rubisco (Osmond, 1978; Farquhar et al., 1989). Both, phase II and phase IV are very sensitive to environmental parameters (Lüttge 2004) and thus the relative contribution of PEPc- (phase II and III) and Rubisco-driven (mainly phase IV) discrimination might also vary with the environment."

Section 3.1 could be reduced in length. It is stated above (Page3632/line27) that phloem transport probably does not change the isotopic composition of carbon compounds. Hence, a long description of phloem transport is not really needed in this context, although scientifically interesting.

→ We think that the topic of phloem transport is central for understanding carbon allocation and carbon isotope fluxes in the plant-soil-atmosphere continuum. We have now made more clear why the understanding of transport processes and the underlying processes is crucial to interpret the carbon and carbon isotopic dynamics on the ecosystem scale:
"The process of C transport in the plant itself is not assumed to fractionate against the 13C-isotopologues of the transported compounds. However, temporal changes in C allocation and metabolic processes along the transport pathways can strongly affect this relationship between environmental conditions and δ13C. For example, it has been observed that phloem sucrose is 13C-enriched in the trunk compared to the twig phloem of trees (e.g. Brandes et al. 2006; 2007). It is likely that metabolic processes associated with phloem transport but not the transport (such as phloem loading or phloem transport; 5 in Fig. 1) itself is responsible for these patterns. Since phloem-allocated sucrose is the main carbon source for all processes in non-green plant parts, spatial variations in δ13C along the plant axis and the processes involved need to be taken into account when interpreting respiratory isotope signals. Moreover, transport dynamics determine the coupling of the isotope signals above- and belowground and thus an understanding of the underlying processes is crucial to interpret carbon isotope signals on the ecosystem scale."
We now also refer more detailed to the hypothesised mechanisms, which is responsible for the continuous ^{13}C enrichment of phloem transported sugars in basipetal direction. This mechanism

is not directly due to phloem transport itself but closely linked to the general principles of phloem transport. We now state:
"During transport, sugars are released from the sieve tubes and part of them is retrieved again (Minchin and Thorpe, 1987). This mechanism of carbon release and partial retrieval might also explain the often observed ^{13}C enrichment of phloem sugars during transport in basipetal direction (Gessler et al., 2009b). Part of the sugars released might undergo metabolic conversion in reactions fractionating against the heavier isotopologue. Due to mass balance reasons the unreacted sugars, which are reloaded in the phloem, will be ^{13}C-enriched (Hobbie and Werner, 2004)."

In section 3.3, plant losses via respiration and BVOC emissions, there's no mentioning of the use of stable isotopes associated to works on the emissions of BVOC. It would be very interesting to the reader to include some knowledge and perspectives on this topic.

→ We have now added information about stable isotopes in BVOC in a separate paragraph.

Page3646/line24: It is suggested to make this a separate section on SI methodologies and elaborate somewhat more on the content including e.g. specific characteristics of the "new cutting-edge technologies" as compared to conventional IRMS. Some mentioning of new technologies in the Conclusions and outlook (P3663/line17..) could be merged into this section.

→ We have made a separate section (new section 6) on stable isotope methodologies, following the reviewer's suggestions.

In section 4.3 on fractionation due to microbial metabolism there's no mentioning of the potential role of autotrophic microorganisms in the context of SMC C-13 signals. Fractionation in autotrophic bacteria has been reported to be interestingly high, see eg. Cowie et al., 2009, Organic Geochemistry. The text reads easy in many places, but in others it is characterized by relatively long sentence constructions. Please, consult once again for linguistic corrections to polish and make more fluent.

→ We added a paragraph mentioning isotopic fractionation in context with autotrophic and photoautotrophic C fixation:
"Furthermore, autotrophic and photoautotrophic CO_2-fixation must be considered in terms of C fractionation. On the one hand, the 3-hydroxypropionate pathway causes smaller isotopic fractionation (–13 to 14‰) compared to the Calvin cycle (–20 to –25‰, van der Meer et al., 2007, and references therein), which is of special importance in systems where microbial mats and cyanobacteria play a large role in C translocation to soil and soil microbial biomass. On the other hand, autotrophic organisms may express a high level of isotopic fractionation, and fractionation has been reported to be interestingly high within the context of inorganic C fixation (Cowie et al., 2009)."

P3623/line9 vs. line20: The terms 'global change' and 'climate change' are often used synonymously in the literature, and also here. However, increasing atm. CO2 is (supposedly)

a driver of climate change, and not a climatic variable undergoing changes and needs to be considered as an element in global change.

→ We have changed the sentence to "…predicted climate and atmospheric composition changes…"

P3624/line13: Insert "atmosphere-plant-soil" interactions

→ Done.

P3626/line1: Must be a "simplified model of Eq. (1)"

→ Corrected.

P3627/line16: Was this a Northern hemisphere study so that June and July refer to summer; please, specify.

→ Has been specified („at a field site in France")

P3632/line3: It is unclear what is meant by "respired CO2" in the discussion on isotopic signatures in different plant organs. Is it respiration from the organs? Please, specify.

→ We have now removed "respired CO_2" in this sentence because the differences in isotopic signatures described here only refer to different plant organs, not to respired CO_2.

P3645/line15: Do the finding imply that time lags decreased with increasing or decreasing plant height? Please, specify.

→ Time lags decreased with increasing plant height in grasses. We have specified that in the respective sentence: "Furthermore, also time lag studies in grasses need to be considered independently, as – in contrast to tree species – time lags may even decrease with increasing plant height as has been shown for *Lolium perenne* (Kuzyakov and Gavrichkova, 2010)."

P3645/section3.6: It is suggested to swap the two sections 3.6 and 3.7 for continuity of description of C transport processes.

→ We followed the suggestion of the referee and swapped the two sections (now sections 3.5 and 3.6).

P3650/line22: It would be very useful to the less experienced reader to include the Rayleigh distillation equation in the text with some additional explanations (e.g. as for the photosynthesis discrimination equation given in section 2.1)

→We included the formula used for Rayleigh distillation in the text. Since the model is not mechanistic in nature, as compared to the Farquhar model for example, we focus on the model's capability to estimate microbial fractionation in situ, although we note that this estimate is a statically fitted term and inferences based on it are limited.

P3651/line20: Fig. 5 should read Fig. 3.

→ Changed.

P3653/line10: What is meant by carbon fixation by heterotrophs? The term carbon fixation is commonly used for CO2 fixation in autotrophic bacteria. Please, explain.

→ As explained in Miltner et al. (2004), CO_2 fixation can take place by non-photosynthetic microbes and without the need of cycling redox chains (i.e. chemo-autotrophs). This type of fixation by heterotrophs (hence, heterotrophic fixation) is thought to occur through anaplerotic reactions (e.g. Krebs cycle).

P3656/line17: Presumably this section deals with aboveground litter, but dying roots should also be considered as litter belowground. Please, specify.

→ We included the word "leaf" with all reference to the word litter in this section.

Page3656/line24: The differential C-13 signals of heterotrophic vs. autotrophic plant organs (described section 2) and their possible differential turnover times might potentially also affect the isotopic composition of the litter layer. This could be further discussed in this section.

→We agree with this comment and have added the following sentence to clarify this point: "To a large extent, the isotopic composition of leaf litter is determined by the plant organs and tissues deposited as well as the post-photosynthetic fractionation that occurred during their synthesis (see section 2.2). For example, roots and woody stems are generally enriched in 13C when compared to leaves and the isotopic signature of organic matter in the litter layer is often close in value to the isotopic composition of aboveground plant organs (Badeck et al. 2005). Thus, the different 13C signals of heterotrophic and autotrophic plant organs and their turnover times may affect the isotopic composition of the litter layer. Scartazza et al. (2004) found no significant variation in δ13C of litter layer, in a beech forest in the central Apennine Mountains, Italy, when there was a significant seasonal change in δ13C values in leaves and in phloem sap sugar. In the study by Scartazza et al. (2004), there was a significant relationship between leaf sugar δ13C and ecosystem respired 13CO2. Thus, the different 13C signals of heterotrophic and autotrophic plant organs may control 13CO2 produced from the ecosystem for some extent, but in terms of litter layer 13C, other C sources may be determining the δ13C of litter layer (e.g. lipids), and C with a short turn over time (e.g. sugar) may not influence the δ13C of litter layer.

P3658/line18: Please, give some more details on “stable isotope probing”.

→ We have amended the sentence with a short explanation of stable isotope probing: “Through stable isotope probing (SIP), i.e. detecting and quantifying isotopic tracers in DNA of the organisms of interest, it is now possible to characterize microbial communities utilizing carbon from ^{13}C-labeled litter or continuous ^{13}C-labeling.”

P3659/line10: A small 1-2 sentence summary of section 4.5 emphasizing formulation of open research questions could be added, as for previous sections.

→ We added the following paragraph:
“Leaf and root litter are important links coupling the short- and long-term carbon cycles belowground, and many open questions remain in resolving their dynamics. Of particular interest will be the fate of organic molecules derived from plant litter as they travel through the many branching points belowground. How these molecules vary spatially and temporally and whether or not they are available as substrate or physically occluded in the soil matrix are other challenges to elucidating plant–soil interactions. Isotopes will remain an important tool in tracing the carbon continuum, especially with the advent of new tools that give higher resolution spatially, for example nanoSIMS, and temporally, for example infrared laser absorption spectroscopy (section 6).”

Fig. 2: There’s no reference to Fig. 2 in the text. Please, insert.

→ We have made reference to Fig. 2 in the text and modified the respective paragraph:
“In certain studies, seasonal changes in belowground C allocation had no effect on the time lag between assimilation and use of assimilates in belowground respiration (Horwath et al., 1994; Högberg et al., 2010), suggesting that phloem path length and structural differences were the main determinants of C transfer velocity. In contrast, other studies reported considerable variation of the time lag during the growing season in the same trees (Plain et al., 2009; Wingate et al. 2010; Kuptz et al., 2011a) (Fig. 2). However, the mechanisms behind such variability are still unknown even though seasonal variations of carbon storage and remobilization in the trunk are the most likely mechanisms to affect the transfer of carbon as well as the conveyance of the carbon isotope signal from the canopy in basipetal direction over the growing season (Offermann et al. 2011).”

Fig. 3: This figure summarizes interesting information, but is difficult to understand. The figure text needs further elaboration in order to identify the respiration processes involved in data compilation. Plant respiration, ecosystem respiration or soil respiration.

→ We further clarified the figure by specifying the estimates as apparent fractionation:
“Estimates of apparent fractionation associated with soil microbial respiration determined by different experimental approaches (grey bars: from C_3 plants; dark grey bars: Rayleigh distillation methods, black bars: C_4 plants).”

Fig. 4: This is a nice figure. To compile current knowledge even further, it is suggested to insert some typical fractionation factors in the grey boxes to illustrate the importance.

→ As we found that very difficult, we refrained from adding fractionation factors to Fig. 4. On the one hand, this figure is already very rich in information. Thus, adding more information would have easily overloaded the Figure. On the other hand, we might have given ranges for particular fractionation factors, but they are in many cases large. Some factors are known quite well, others are estimated only theoretically. Actual fractionation in situ might not related to theoretical kinetic isotope effects due to several reasons (see Werner et al., 2011). If we had included fractionation factors we would have needed to give an additional and extensive explanation of the figure elsewhere.