

**Sediment source attribution from multiple land use systems with CSIA**

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# Sediment source attribution from multiple land use systems with CSIA

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## Abstract

As sediment loads impact freshwater systems and infrastructure, their origin in complex landscape systems is of crucial importance for optimization of catchment management. We differentiated sediment source contribution to a lowland river in Central Switzerland in using compound specific stable isotopes analysis (CSIA). We found a clear distinction of sediment sources originating from forest and agricultural land use. We suggest to generally reduce uncertainty of sediment source attribution, in (i) aiming for approaches with least possible data complexity to reduce analytical effort as well as refraining from undetected source attribution and/or tracer degradation obscured by complex high data demanding modelling approaches, (ii) to use compound content (in our case long chain fatty acid (FA)) rather than soil organic matter content when converting isotopic signature to soil contribution and (iii) to restrict evaluation to the long-chain FAs ( $C_{22} : 0$  to  $C_{30} : 0$ ) not to introduce errors due to aquatic contributions from algae and microorganisms. Results showed unambiguously that during base flow agricultural land contributed up to 65 % of the suspended sediments, while forest was the dominant sediment source during high flow, which indicates that during base and high flow conditions connectivity of sediment source areas with the river change. Our findings are the first results highlighting significant differences in compound specific stable isotope (CSSI) signature and quantification of sediment sources from land uses dominated by C3 plant cultivation.

## 1 Introduction

The United States Environmental Protection Agency has identified sediments among the top ten causes of biological impairment in freshwater ecosystems (US EPA, 2009). On an European perspective, sediment pollution has been identified as one of the most relevant pressures to water bodies which will impede to achieve the aims of the water framework directive by the year 2015 (Borja et al., 2006). Restoration of rivers from sed-

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## 2 Materials and methods

### 2.1 Site description

The river Enziwigger is a small and canalized river located in the Canton Lucerne, Switzerland, near Willisau, with a watershed size of 31 km<sup>2</sup>. The flow regime at the sampling sites is not affected by any hydro-power or waste water treatment plants. The ecomorphology of the river has been strongly modified and currently only 5 % is close to natural. Terraces have been installed to prevent deep channel erosion and scouring of the bed during flood events. Three experimental sites A, B, and C (from up- to downstream, see Fig. 1) were installed at altitudes of 757, 625 and 583 m above sea level, respectively. For complete experimental setup and additional study site information please see Schindler Wildhaber et al. (2012b).

### 2.2 Suspended sediment sampling

Suspended sediments were sampled at three sites A, B and C along the river (Fig. 1), with site A being near the headwaters of the catchment under forested and pastured land covers, while river sections at site B and C are potentially influenced by pastures (C3 grasses only), forest (mainly coniferous) and arable land (mainly wheat production, some maize in single years but with no detectable effect on stable isotope signature of soils, Schindler Wildhaber et al., 2012a). We consider river bank not an original separate source to river sediments since we either have a continuum of forest or grassland soils down to the river banks or small grassland river banks act as intermediate deposits to sediments from source soils. Further, we did not include riverbed in our analysis, since riverbed sediments themselves (e.g., the underlying bedrock) should not influence the CSSI signal, assuming the fraction of petrogenic organic carbon to be low with no significant contribution in FAs to the sediments. The latter might be a source of error for storm flow events but most likely not for base flow conditions with low sediment contribution (Galy et al., 2015). If riverbed material contain biospheric FAs,

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these should be either originating from terrestrial sources which in our analysis will be attributed to the original source or should be of aquatic origin which means we cannot separate them from the riverine FA production not connected to sediment transport.

Suspended sediments (SS) were collected at the three sites with time-integrated SS-samplers after Phillips et al. (2000). They were emptied in a weekly interval. For more detailed information see Schindler-Wildhaber et al. (2012b).

Water level at the three sites was measured in 15 s intervals with pressure transmitter probes (STS, Sensor Technik Sirnach, Switzerland). Average values were logged every 10 min. For detailed experimental setup see Schindler-Wildhaber et al. (2012b).

## 2.3 Soil sampling

Upstream of each of the three sites A, B and C, representative soil samples of each land use type forest, pasture and arable land were taken, each of them sampled in triplicates (see Fig. 1 for the location of the source area sampling sites). For forest sites, the humus layer was removed prior to sampling. The upper 5 cm of the topsoil were sampled with a cylindrical steel ring (98.2 cm<sup>3</sup>) and then stored in plastic bags.

After collection, soil samples were stored in a fridge at 4 °C. For analysis of carbon and nitrogen contents in the soil and SS, the samples were oven-dried at 40 °C for at least 48 h, roughly ground in a mortar, and stones as well as root material were removed. The samples were ground with a ball mill (Retsch MM400, Retsch GmbH, 42781 Haan, Germany) for 90 s at a frequency of 24 s<sup>-1</sup>.

## 2.4 Carbon and nitrogen analysis

The milled samples were analysed for organic and inorganic carbon as well as for nitrogen contents. Total nitrogen was measured with a LECO CN628. Total organic carbon (TOC) and total inorganic carbon (TIC) were analysed on a LECO RC612 (LECO, St. Joseph, Michigan 40985, USA).

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## 2.5 Lipid extraction and preparation

Soil samples (11–21 g) and SS (4.5–25 g) were extracted after the method of Elvert et al. (Elvert et al., 2003). For quality and quantification control an internal standard with known concentration and  $\delta^{13}\text{C}$  isotopic value, nonadecanoic acid, was added to the samples prior to extraction. To monitor the quality of lipid extraction batches and analysis performance, one sample (pasture at site C) was extracted in each extraction batch ( $n = 3$ ) and further analysed.

Extraction was done by ultrasonication of the soil and sediment samples, which were put in PTFE centrifuge tubes, using solvent mixtures of declining polarity. First 25 mL of methanol(MeOH)/dichloromethane(DCM) (2 : 1,  $v/v$ ), followed by MeOH/DCM (1 : 1,  $v/v$ ) and two steps with pure DCM were used for the ultrasonic extraction. In between the ultrasonication steps, the PTFE tubes were centrifuged (5 min at 4000 rpm,  $0^\circ\text{C}$ ). The supernatant was pooled in a separation funnel and partitioned against pre-extracted 0.05 M KCl solution. The organic phase at the bottom of the funnel was collected and evaporated under a stream of nitrogen. This resulted in the total lipid extracts (TLE). Half of the TLE was removed and stored as backup in the freezer at  $-20^\circ\text{C}$ . The other half was transferred to a 5 mL reaction vial and 1 mL of 12 % KOH in MeOH for saponification was added. Saponification was maintained at  $80^\circ\text{C}$  for 3 h. After cooling down 1 mL of 0.1 M KCl was added. The neutral lipid fraction was then extracted from the basic solution by agitating 4 times with ca. 2 mL hexane, dried under a stream of nitrogen and stored in the freezer at  $-20^\circ\text{C}$ . The remaining solution was set to pH 1 with concentrated HCl. Free FAs were extracted by again agitating 4 times with ca 2 mL hexane. The extract was also dried under a stream of nitrogen and then 1 mL of 12–14 %  $\text{BF}_3$  in MeOH was added. Methylation reaction of free FAs to fatty acid methyl esters (FAMES) took then place at  $60^\circ\text{C}$  for 1 h. A last hexane extraction step as above in presence of 1 mL 0.1 M KCl was performed. The final extract was evaporated under a stream of nitrogen and stored in the freezer at  $-20^\circ\text{C}$ . Samples were extracted in three different extraction batches. To monitor the quality of lipid extraction and analy-

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sis performance, one sample (Pasture source site C) was extracted in each extraction batch and further analysed.

## 2.6 Gas chromatography and isotope ratio mass spectrometry

Concentrations of FAMES were determined by using a Trace Ultra gas chromatograph (GC) with a flame ionization detector (FID) (Thermo Scientific, Waltham, MA 02451, USA). GC oven temperature started at 50 °C and was increased to 150 °C at a speed of 10 °C min<sup>-1</sup>, hold for 1 min, increased to 300 °C at a speed of 4 °C min<sup>-1</sup> and hold for 63 min. The carrier gas helium was set to a constant flow of 1 mL min<sup>-1</sup>. Injector temperature was set to 300 °C and the detector temperature to 320 °C. Concentrations of FAMES were calculated relative to the internal standard nonadecanoic acid, which was added prior extraction.

The FAMES were identified using the same Trace Ultra GC as above, coupled to a DSQ mass spectrometer (Thermo Scientific). The GC-MS is equipped with the same injector and capillary column and uses the same method as described above. Transfer line temperature to MS was set to 260 °C. Carbon isotopical compositions of the FAMES were analysed using a Trace Ultra GC coupled via combustion interface GCIsolink and ConfloIV with a DeltaV Advantage isotope ratio mass spectrometer (Thermo Scientific). The system is equipped with a split/splitless injector, operated in splitless mode. The combustion oven was set to 1000 °C. GC oven temperature started at 50 °C and was increased to 140 °C at a speed of 10 °C min<sup>-1</sup>. Then it was hold for 2 min and increased to 300 °C at a speed of 4 °C min<sup>-1</sup> and hold for 35 min. The carrier gas helium was set to a constant flow of 1.2 mL min<sup>-1</sup>. Injector temperature was set to 300 °C. Carbon isotopes were reported in delta notation, per mil deviation from Vienna Pee Dee Belemnite (VPDB). The system was externally calibrated with Schimmelmann Std B3. Performance has been controlled with a C19:0 FA internal standard. The reported  $\delta^{13}\text{C}$  values have been corrected for the additional carbon atom introduced during methylation and had an analytical uncertainty lower than  $\pm 0.5\%$ .

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## 2.7 Procedural error and measurement precision

Measurement precision of the GC-IRMS is 0.5‰. However, considering the analytical uncertainty only (e.g., checking an externally added standard) might neglect uncertainties, which bias the interpretation of isotope data. We recommend analysing single samples in multiplicities as procedural controls to estimate the reproducibility within the analysis procedure and the heterogeneity in one sample bag. We analysed three times a sample including lipid extraction (pasture, Site C) and resulted in an overall procedural standard deviation of 0.13, 0.84 and 0.26‰  $\delta^{13}\text{C}$  for C14:0, C26:0 and C28:0 FAs, respectively.

For assessment of the source heterogeneity, we present the standard deviation of the different sampling spots within our source areas (Table S1 in Supplement). To establish mixing lines for sediment source attribution we calculated mean values of source areas (Figs. 2–3). Deviation of SS from the mixing line should not be greater than procedural error or measurement precision otherwise contribution of additional sources cannot be excluded.

## 2.8 Unmixing of suspended sediment signatures

Deducing from mathematical constraints, it is possible to find unique algebraic solutions for the sediment source attribution with  $n$  tracers for  $n + 1$  sources resulting in an equation system with  $n + 1$  equations and  $n + 1$  unknown variables. Mixing models like IsoSource (Phillips and Gregg, 2003) or, more recently, Bayesian mixing modelling (e.g., Smith and Blake, 2014; Cooper et al., 2015a) have been employed to establish confidence intervals around the estimates. Mixing models like IsoSource (Phillips and Gregg, 2003) relax the strictly linear system and allow for multiple solutions but without explicit incorporation of source and suspended sediment variability. The multiple valid solutions to the linear system produced by IsoSource can be plotted in a histogram-like fashion, although unlike Bayesian models they do not represent probability distribu-

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cannot exclude a small contribution from an additional source and/or slight degradation of the signal during transport.

Because of the non-significant difference between the CSSI signatures of pasture and arable land (Fig. 3, left), we can solve for the sediment contribution at sites B and C also only from two different sources if we want to remain statistically firm: forest versus agricultural land (the latter bulking the signals from pasture and arable land). The same algebraic solution was used as for site A, correcting suspended sediment isotope signals of both FAs on the mixing line of sediment sources.

Aggregating the data from the land use types pasture and arable land is useful, not only because of the non-significant difference between the sources but also because the combined source group has a functional significance (agricultural versus forest land use). However, a separation between pasture and arable soil sources might seem desirable from catchment management perspectives. If we want to separate between pasture and arable land using the non-significant source signal differences of C26:0 and C28:0 as tracers, the mixing model IsoSource is useful. IsoSource constrains the relative proportions of the various sources in the mixture by evaluating all possible combinations of each source contribution (from 0–100%). Even though we used the model to calculate sediment source contribution from all three sources (Table 1), we are fully aware that the separation between pasture and arable land cannot be considered as statistically firm. The latter also implies that the application of a more complex Bayesian mixing model seems meaningless.

The only FA resulting in significant differences between tracer signals of soils from the two land use types pasture and arable land was the C14:0 FA (Table S1). However, using this FA as a tracer did not lead to meaningful solutions (e.g. negative sediment source contributions), because the isotopic values of the sediment mixture (suspended sediments) are not within the isotopic values of the source endmembers (Fig. 3 right). No set of source proportions is possible if the isotope mixture of the suspended sediments is outside the convex polygon bounded by the sources (Phillips and Gregg, 2003). Short-chain and medium-chain FAs (C12:0 to C16:0) are mainly not produced

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by higher plants but by microorganisms and algae, mainly by aquatic algae (Hughen et al., 2004; Freeman and Pancost, 2014). As such, the FA signals we determined in the suspended sediments were most likely influenced by aquatic contribution as an additional source. The latter is confirmed by the generally higher concentrations of C14:0 FAs in our suspended sediments compared to source soils as well as in base flow SS compared to high flow SS (Table S1), which indicated the riverine origin. Thus, even though short-chain and medium-chain FAs have been used to track terrestrial sediment contribution to rivers (Gibbs, 2008; Blake et al., 2012; Hancock and Revill, 2013) we would highly suggest constraining the concept of tracking terrestrial sediments to the long-chain FAs (C22:0 to C30:0).

Because the CSIA method traces carbon rather than the soil itself, the results given by the unmixing of the  $\delta^{13}\text{C}$  signals need to be adjusted to account for the different amounts of each of the soil sources. Following solutions in recent literature the percent carbon content of each source was used to weight sediment source attribution (Gibbs, 2008; Hancock and Revill, 2013; Blake et al., 2012). However, the relative carbon distribution in each source might be very different than the relative distribution of the specific tracer FA (Fig. 4). Since we used specific FAs as tracers and not the total soil organic carbon, we corrected with the concentration sum of the respective FAs (see methods). The difference between these two correction approaches might be considerable. In our study, a correction with soil organic carbon content would overestimate forest contribution and underestimates arable land up to 13%. However, depending on the site-specific differences in the relation of soil organic carbon to specific FA content, the uncertainty introduced might be even higher at other study locations. Further, if quality and characteristics of bulk SOC is different between sources, degradability during detachment and transport might also be very different which will increase uncertainty if correction is carried out with bulk SOC. Thus, we highly recommend for future CSIA studies to correct with the sum of FA content and not with the soil organic matter content.

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IsoSource modelling at sites B and C from pasture are 20–30 % during base flow and 5–20 % during high flow and from arable land 45 % during base flow and 10–30 % during high flow. However, these separations within the agricultural land uses should be regarded with caution, as tracer signals of sources are not significantly different.

As rivers are slowly but progressively recovering from the effects of acidification, eutrophication and pollutant contamination (Alewell et al., 2000, 2001; Palmer et al., 2010; Layer et al., 2011), the expected increase of sediment input to rivers in the future is an unsolved problem (Matthaei et al., 2010; Scheurer et al., 2009). Without assessing sediment sources and their connection to different land use types, catchment management will be impeded to make progress in sediment load reduction. Because of the work and cost intensive analytical procedures, CSIA might be far from being used as a regular management tool. Nevertheless, it might give insight into sources of sediments in some selected studies. Furthermore, as we have seen with the rapid improvement of analytical tools in recent years, CSIA has the potential to develop as an important tool for highly selective point measurements, where sediment origin and thus catchment management options are unclear. As such, focus of research development should be directed towards biomarker tracer approaches with least possible analytical effort using low numbers of tracers set up for straight forward iso-space evaluations.

## 4 Conclusions

Our aim was a rigorous sediment source attribution with CSIA of fatty acids from three different land use types (forest, pasture and arable land) dominated by C3 vegetation only. We achieved significant differences between forest and agricultural soil sources for four of the investigated fatty acids (C18:0, C22:0, C26:0 and C28:0 FAs). Only one fatty acid (C14:0) resulted in significant differences between pastures and arable land, but a discrimination within these two agricultural sources was not possible, because results indicated a likely influence of aquatic contribution to the CSSI of this low chain fatty acid. We recommend using long chain fatty acids (C22:0 to C30:0) only for sedi-

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ment source attribution from terrestrial sources. We further would like to suggest using compound content (in our case long chain fatty acid content) rather than soil organic matter content when converting isotopic signature to soil contribution.

Sediment source attribution resulted in high sediment contribution from forests during high flow conditions but domination of sediment input from agricultural sources during base flow. Thus, connectivity of sediment source areas with the river changed with changes in flow regime.

Catchment managers are often called to make soil conservation decisions on the basis of land use, as different land use types are connected to differences in soil erosion severity. Assuming the CSIA to develop further to a routine analysis in the future, it might become a valuable decision tool as a sound and scientifically accepted proof to track down sediment origin. We would like to recommend setting the research focus in the near future on developing sediment source attribution biomarker approaches with low tracer numbers aiming at unique mathematical solutions, thus optimizing analytical efforts and reducing uncertainty.

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*Author contributions.* Christine Alewell provided the project idea and the concept of the study and took the initiative to start the project. She contributed to the data interpretation and had the leading role in manuscript writing. Katrin Meusburger contributed most to data evaluation, was responsible for the IsoSource modelling and contributed to the manuscript writing. Axel Birkholz took the lead in method development and CSIA, but was also part of data evaluation and manuscript writing. Yael SchindlerWildhaber developed and implemented the field study and sampled the suspended sediments. Lionel Mabit was involved in data interpretation, discussion and manuscript writing.

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duced by permission of Swisstopo (BA15012). CSIA was based on methods and analytical equipment Helge Niemann established in our labs. We thank Elena Frenkel for mathematical help with regression correction.

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**Table 1.** Contribution of the different sediment source areas to the SS, calculated with the different methods and using two or three sources and two FAs as tracers (i.e. C26:0 and C28:0). Values in brackets represent the uncertainty ranges of the estimates.

Site	Event	2 Tracer/2 Sources		2 Tracer/3 Sources (IsoSource)		
		% Forest	% Agriculture	% Forest	% Pasture	% Arable
A	BF	70.2 (40–100)	29.8 (0–47)			
A	HF 2010	85.0 (54–100)	15.0 (0–37)			
A	HF 2009	59.7 (31–92)	40.3 (12–55)			
B	BF	36.7 (12–60)	63.3 (51–72)	28.2* (25–48)	16.6* (0–56)	55.2* (0–75)
B	HF 2010	93.5 (76–100)	6.5 (0–24)	92.1 (90–100)	2.4 (0–8)	5.5 (0–10)
B	HF 2009	78.1 (59–100)	21.9 (0–41)	69.5 (61–93)	9.4 (0–31)	21.1 (0–39)
C	BF	34.3 (15–57)	65.7 (33–79)	31.8 (38–58)	23.6 (0–56)	44.6 (0–62)
C	HF 2010	71.5 (53–100)	28.5 (0–37)	64.7 (67–93)	12.3 (0–29)	23.0 (0–33)
C	HF 2009	54.7 (35–85)	45.3 (10–55)	49.2 (52–80)	17.7 (0–42)	33.1 (0–48)

HF = High flow

BF = Base flow

\* For BF sediment contribution at Site B a unique solution was possible.

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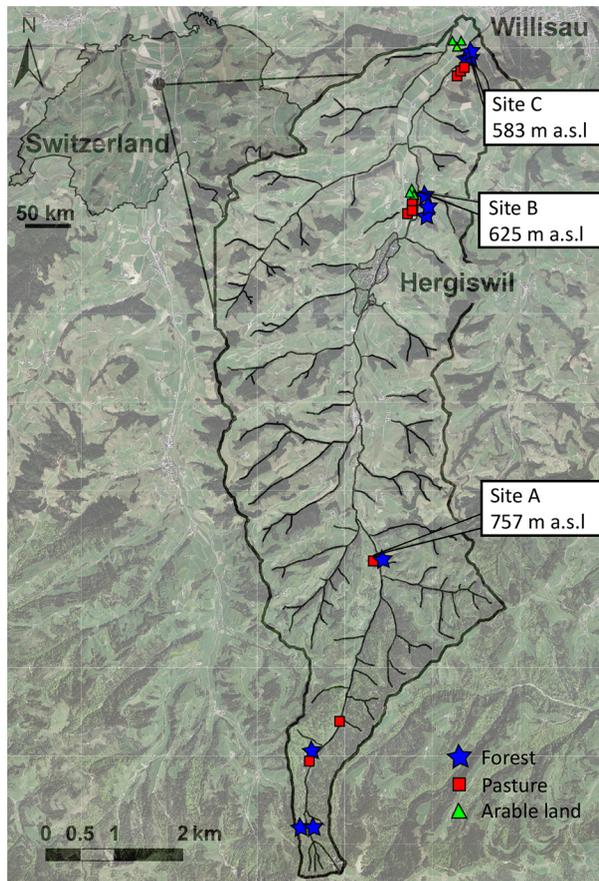
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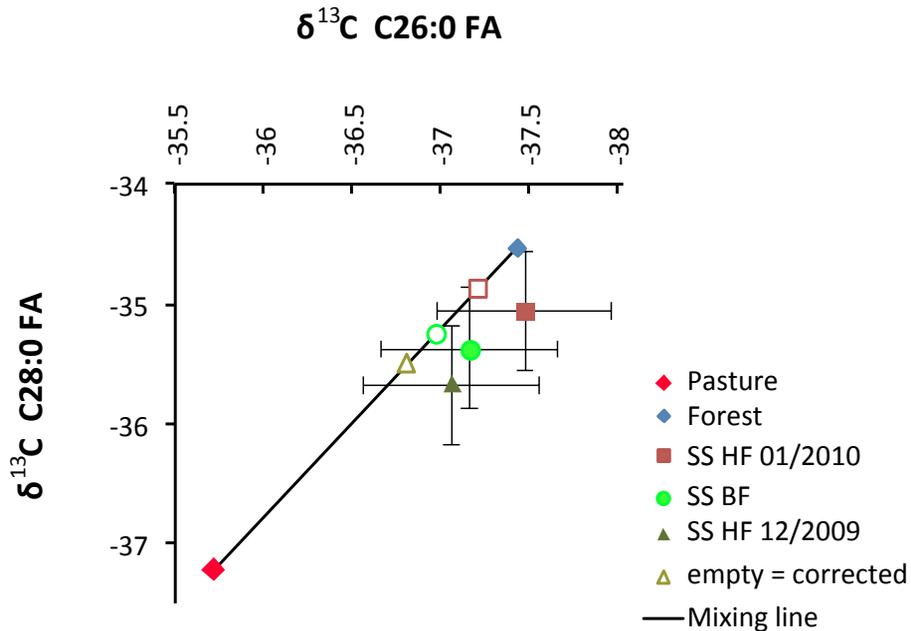
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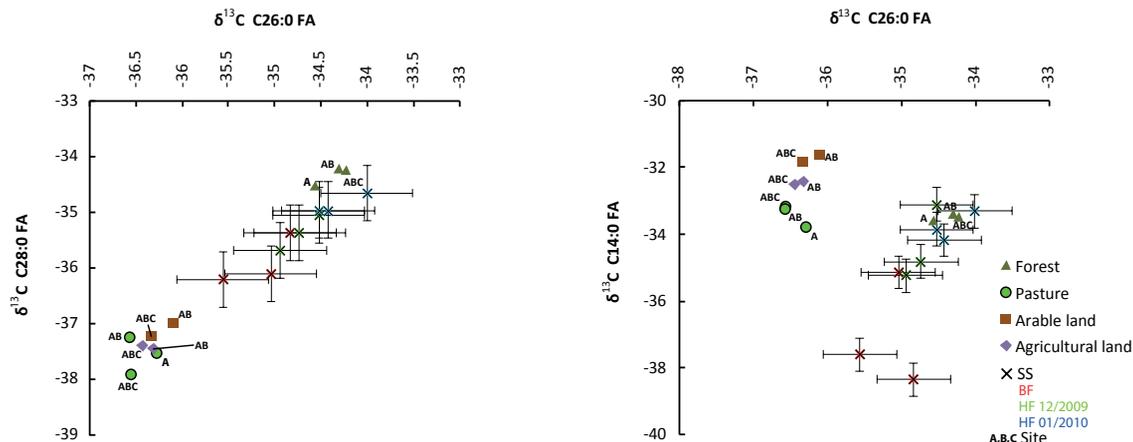
**Figure 1.** The Enziwigger catchment (Canton Lucerne, Switzerland) with the three suspended sediment sampling sites A, B, C and location of the source soil sampling spots forest, pasture and arable land.



**Figure 2.**  $\delta^{13}\text{C}$  of the FAs C26:0 and C28:0 in suspended sediments (SS) of two high flow (HF) and one base flow (BF) events and the two possible sediment sources from land use types pasture and forest at site A. Considering measurement un-precision,  $\delta^{13}\text{C}$  were corrected to the mixing line with linear regression. Error bars of SS display the procedural error of 0.5%.

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**Figure 3.**  $\delta^{13}\text{C}$  isotopic signatures of FAs C26:0 versus C28:0 (left) and C26:0 versus C14:0 (right) of sediment sources and suspended sediments (SS) at the three sites (A, B and C) in the Enziwigger catchment. Error bars of SS display the procedural error of 0.5‰.

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