Reply to Anonymous Referee #1

Reviewer 1 mainly addresses three points: 1) neglect of literature, 2) unnecessary reanalysis of samples of Schindler et al., 2012 and rationale of the introduction in general, and 3) use of terminology. We would like to address these three main points in the first paragraph while we will answer to the more specific comments in the section below.

Regarding the first point of critique, reviewer 1 feels we neglected relevant literature. Thanks for the effort to provide us with a list of studies on CSIA on soils, organic matter and sediments. We carefully evaluated all suggested studies but we think many are actually not relevant for this manuscript or redundant to the studies we already quoted. Our aim here is not to write a general review on the use of CSIA in soil science or on the use of CSIA on organic matter transport from terrestrial sources to marine sediments. Our focus was on studies that used sediment source fingerprinting with CSIA in fluvial systems. Glaser et al., 2005, Jandl et al., 2000, Madan et al., 2002, Naafs et al., 2004a, Naafs et al., 2004b, Nierop et al., 2001 and 2005 are studies on CSIA generally in soil science or on CSIA of SOM and do not contribute to the topic of our manuscript. Colombo et al. 1997, Jeng and Huh, 2004; Sanchez-Garcia et al., 2008 investigate terrestrial versus petrogenic and/or aquatic origin of organic matter in marine sediments. Colombo et al., 1997 discusses possible degradation of FAs, but we evaluate the isotopic signature of FAs and not their content. As such, the question is whether or not this signature is altered if there is degradation and not if we have a change in the absolute content. The latter is addressed by some of the other studies the reviewer mentioned, generally supporting the idea that there is no fractionation of compound-specific stable isotope (CSSI) of FAs during degradation and/or that FAs are remarkable stable (Drenzek et al., 2007; Marseille et al., 1999; Wiesenberg et al. 2004). While Drenzek et al., 2007 and Marseille et al., 1999 find FAs to be more stable than alkanes, Wiesenberg et al. 2004 finds alkanes to have longer turnover times than FAs. There is more literature to support our approach of using long chain FAs as tracers of terrestrial sediments and some of them point out that the short chain FAs are of bacterial and/or aquatic origin (Eglinton and Eglinton, 2008; Ficken et al., 2000, Huang et al., 1996; Lichtfouse et al., 2004, van Dongen et al. 2000). Of course we could add some of these to the already quoted studies in our manuscript. The same applies to studies investigating the change from C3 to C4 plants: Ficken et al., 2002; Quenea, 2006.

We are sorry that referee 1 thinks our introduction was very confuse. We revised the introduction considerably and considered the specific comments below during the revision. This overall confusion seems to emerge partly from a misunderstanding regarding the aims of this study, as reviewer 1 thinks it is not useful to analyze CSSI if we already answered the questions regarding the sediments sources in this specific catchment “Enziwigger” with the study of Schindler Wildhaber et al. (2012). Schindler Wildhaber et al. (2012) tracked sediments with bulk analysis of $\delta^{13}C$ which was only possible because nearly all forest cover in this catchment is stocking on calcareous bedrock while all arable land and grasslands are on siliceous material. This is a very rare situation and as soon as we will move on to another catchment this approach will not be applicable any more. As such we used the setting to test whether or not the CSSIs of fatty acids might be suitable tracers which are also suitable for catchments with no change in geology between the different land cover/land uses. In contrast to the reviewer we don’t consider the analysis of Schindler Wildhaber et al. (2012) and our analysis redundant since the results found by Schindler Wildhaber et al. (2012) can be used to verify the suitability of CSSIs of fatty acids as sediment fingerprints in C3 plant dominated catchments. When revising the respective parts throughout the manuscript. A further aim was to test if we would find a difference in the CSSI signatures of FAs from grassland and arable soils which of course is not possible with isotope bulk analysis.

We are criticized to mix up the terms CSSI and CSIA. For example, the reviewer thinks we misused the terms in L50-56 (old version of manuscript): "A new technique, using the compound-specific stable isotope (CSSI) signatures of inherent soil organic biomarkers, can discriminate and apportion the source soil contribution from different land-uses in order to reinforce the effectiveness of soil conservation measures (Gibbs, 2008; Blake et al., 2012; Guzman et al., 2013; Hancock and Revill, 2013; Ponton et al., 53 2014). The compound-specific *stable* isotope analysis (CSIA) measures the δ13C or δ2H isotope signature of specific organic compounds associated with the organic matter bound to the soil/sediment."
We cannot see any fault in the use of the terms here. The first sentence addresses the isotope signatures, the second the technique. Maybe the reviewer 1 is "overcritical" here. We admit we accidentally added a *stable* when introducing the technique. As mentioned by reviewer 2 the more logical abbreviation would then be CSSIA, but since previous studies introduced the term CSIA and it is a commonly used term by now, we would rather stick with this abbreviation and deleted *stable* from the term.

Specific Comments :

Introduction:
L34 &38: The use of the word impairment remains unclear "Biological impairment in freshwater" vs "Restoration of rivers from sediment impairment" . Could the authors specify what "impairment " means exactly.
We included: ….sediment impairment (such as clogging of river bed, eutrophication of waters, direct harmful effects of sediments to the biota and destruction of river infrastructure) ….

L40-44: "Geochemical fingerprinting has been used to discriminate between sources of sediments and was successful in discriminating between subsoil and surface soils (Collins et al., 1997; Walling, 2013) but the technique is limited in providing significant differences between sources of different land use types and vegetation cover in complex landscapes (Alewell et al., 2008; Mabit et al., 2013; Mabit et al., 2014; Hancock and Revill, 2013: : :)" References are misquoted: In the paper of Walling, 2013, CSIA is included into the geochemical fingerprint. Alewell et al 2008 deals with carbon mineralization during the soil detachment from the upland to the wheatland. Hancock and Revill 2013, was a paper using CSIA to discriminate land use and vegetation sources. I think that the terminology "Geochemical fingerprint" have to be define to clarify what was its meaning for the authors.
We are sorry for this lapse. The quotes in the second part of the sentence referred to a sentence in an earlier version which we deleted later. Unfortunately in using Endnote the references were not deleted when we deleted the sentence. The latter we did not see when accepting all revisions in the manuscript. Correct are the quotes of Collins and Walling and later in combination with CSIA Blake and Hancock. We actually revised the whole paragraph; please see revised manuscript line 41 – 46.

L45: "If tracer signatures fail to be significantly different sources": Could authors be more explicit. "Tracer signatures" includes a large panel of "geochemical fingerprints" including CSIA that allowed for complex sources determination. L45-49: the paragraph is confused.

Discriminant function analysis has been used with a variety of tracers: elemental composition (studies of Collins et al., Cooper et al.) or CSSI (Smith and Blake) of sources and sediments. To our

understanding it is mainly used if the tracer signatures of the sources are not significantly different and/or if a complex set of tracers was analyzed to identify the most suitable set of tracers. We changed the whole paragraph (line 41-46).

Discussion Paper
L50-56: For "CSSI" the right abbreviation is CSSIs for Compound-Specific Stable Isotopes. The CSSIs being the result of the CSIA= Compound-Specific Isotopes Analysis and not "L54: Compound specific stable isotope analysis". Then the authors should dissociate the "technique" and the fields of applications.
Please see above regarding the use of the terms CSIA and CSSI.

(1) The use of biomarkers such as fatty acids to identify the contribution of organic matter sources to soils and sediments was intensely studied (Colombo et al., 1997; Eglinton et al., 1968; Eglinton & Eglinton, 2008; Jandl et al., 2005; Jandl et al., 2002; Jeng & Huh, 2004; Madan et al., 2002; Marseille et al., 1999; Meyers & Ishiwatari, 1993; Meyers & Takeuchi, 1979; Naafs et al., 2004a; Naafs et al., 2004b; Nierop et al., 2005; Nierop et al., 2001; Perry et al., 1979; Sanchez-Garcia et al., 2008; van Dongen et al., 2000).
(2) The combinaison of biomarkers with stable isotope analysis also called CSIA was also widely used to determine the sources and the fate of organic matter in soils and sediments. (Drenzek et al., 2007; Eglinton & Eglinton, 2008; Ficken et al., 2000; Ficken et al., 2002; Glaser, 2005; Huang et al., 1996; Lichtfouse et al., 1995; Quénéa et al., 2006; Wiesenberg et al., 2004).
(3) The use of CSIA for erosion and catchment management purposes is more recent. I suggest two additional publications on the use of biomarkers and CSIA in suspended sediments (Seki et al., 2010; Shi et al., 2001). Furthermore, the first publication cited by the authors related to the use of CSIA for identifying "soil sources" in estuarine sediment dates back to 2008. We are in 2015. I suggest replacing "New technique" by "recent advances".
I recommend completing the bibliography of the manuscript with some of the publications cited above. The Authors could select the most relevant for their study.

Please see above. We included some of the suggested literature, as pointed out above. But we do not want to include a general review part on CSSI in soils, organic matter and marine sediments or the use of CSIA in soil science and organic matter transfer to the oceans.

L66-72: "In quantitative sediment attribution approaches, the precision of the method was impeded by the non-significant differences in the isotope signals between the different sources (Gibbs, 2008; Blake et al., 2012), especially if organic matter in sediment sources was dominated by C3 plant vegetation (Blake et al., 2012; Cooper et al., 2015b). The latter implied a restriction to (i) differ between sources with vegetation shifts from C3 plants to the warm-climate C4 grasses, which are considerably higher in _13C values: : :" Why non-significant differences in the isotope signals when C3 plant vegetation dominated implied a restriction to differ between sources with vegetation shifts from C3 plants to the warm-climate C4 grasses, which are considerably higher in _13C values. The sentence is confused.

We reformulated the second sentence to
"The difficulty to differ sediment sources from soils from C3 vegetation land cover by CSIA of $\delta^{13}C$ in biomarkers implied (i) a restriction to sources with vegetation shifts from C3 plants to the warm-climate C4 grasses, which are considerably higher in $\delta^{13}C$ values (Ficken et al., 2002; Quenea, 2006; Gibbs, 2008; Hancock and Revill, 2013; Cooper et al., 2015a), (ii) achieving more effective discrimination by including information on $\delta^{2}H$ of *n*-alkanes (Seki et al., 2010; Cooper et al., 2015b) or (iii) including geochemical mineral tracers for the fingerprinting (Blake et al., 2012) which is useful with obvious shifts in geologic bedrock of the soils. " (line 63 – 70).

L91: "reducing method uncertainty in reducing the complexity of the unmixing procedure."
It is the first time the authors introduce "the unmixing procedure". The sentence is difficult to understand, and we don't know "the unmixing procedure" refers to.
The introduction part is very confused. If I resume:
1-Conventional tracers used as geochemical fingerprint failed in differentiating sediment sources when it is too complex (for example several land use types for one catchment). 2- But a new technique, the CSIA allowed for this type of discrimination.
3- Nevertheless, the technique have some limitations: If vegetation coverage have the same phytosynthetically pathway (e.g. C3) the isotopic signal is not significantly different.
4- Finally, to achieve more effective discrimination it is better to include information on D/H of n-alkanes (???) (Question: why did the authors choose to work on FAs), and geochemical tracers for the fingerprint (that corresponds to the (1) of the introduction,)
Authors go round in circles.
Regarding point 3: we did not mean to say that the $\delta^{13}C$ isotopic signal of C3 plants is never significantly different. But previous studies did not find significantly different signatures, which can have various reasons: e.g. imprecision of CSIA, soil heterogeneity, to low sample numbers, changes in land use (former forests might now be grasslands or grasslands might now be arable soils, as such todays source soils might have mixed signals). This is why we used a rather simple catchment setting with only three different land cover types.
Regarding point 4: Previous studies added rather more tracers to tackle the problem, while we chose to go for a simpler system. We are sorry if this was confusing.
Please see our changed paragraph starting in line 88.

Materials and methods.
L176-177: Could the authors precise analitical uncertainties on concentrations.
We specified repetition of samples and measurements, analytical uncertainty and analytical uncertainty. Please see chapters 2.3, 2.5., 2.6. and 2.7.

L194- 196: "However, considering the analytical uncertainty only (e.g., checking an externally added standard) might neglect uncertainties, which bias the interpretation of isotope data" I don't understand the meaning of the sentence.
We explain the difference between procedural error and analytical uncertainty in section 2.7., and we added the following explanation:
"We recommend analyzing single samples in multiplicities as procedural controls to estimate the reproducibility within the analysis procedure (from taking the soil sample out of the sample bag, via the lipid extraction, methylation, identification and quantification of FAs up to the final determination of the CSSI) as well as the heterogeneity in one sample bag."

L192: "We recommend analyzing single samples in multiplicities: : :" I suggest removing the sentence. GC-C-IRMS analyses are always performed in replicate as conventional procedure in all serious laboratories.

We think this is a misunderstanding. We refer here to the procedural error not the measurement precision, please see above.

Results and discussion

The discussion on multiple sources of fatty acids in sediments is very week. Your suggestion to constrain the track of terrestrial sediments to n-alkanoic acids > n-C22, is already largely recognize, See (Meyers & Takeuchi, 1979; Pearson & Eglinton, 2000; Shi et al., 2001, Galy et al., 2011), and references cited above. Furthemore, Authors cited Galy et al. 2011, and in this paper, it could be notice that only the FAs from C24 to C32 were used to track terrestrial sources in sediments. Short chain alkanoic acids are characteristics for algae, bacteria, aquatic microflora and microorganisms (Boon et al. 1975; Perry et al. 1979; van Vleet and Quinn 1979; Volkman 1986, Banowetz et al. 2006). I think that there is a confusion between the use of FAME microbial soil profiles as soil geochemical fingerprints in surface waters and the use of terrigenous FAs as tracers of vegetation and land use in sediments for erosion purpose. I also observed this confusion in Gibbs, 2008 and Blake et al., 2012.

We track soil sediment transport to rivers. As such we are referring/ comparing to other studies with the same aim (Gibbs, 2008; Blake et al., 2012 as well as Hancock et al. 2013). And we noticed, as you did too, that Gibbs and Blake et al. used the short chain fatty acids to track terrestrial sediments. Pearson and Eglinton, 2000 are actually looking at $\Delta^{14}C$ and $\delta^{13}C$ of long-chain n-alkanes ($C_{24\text{-}33}$) from ocean sediments. The data were then simulated using a three-component mixing model designed to represent the contributions of the different sources (petroleum, modern plant wax, and shale-derived alkanes) of organic matter in the sediments. So this is actually a very different scientific community and of course they are aware of short chain alkanes being connected to bacterial or algae origin. The same holds true for Galy et al., 2011 or Shi et al., 2001. But the scientific community tracking terrestrial soil sediments (soil erosion community) is obviously not aware of the problem and most likely they will be reading our paper and less so the ocean community.

We could not find Meyers & Takeuchi, 1979. Do you refer to Takeuchi and Meyers, 1976?

These are the reasons that lead previous reviewers to reject the manuscript. When they asked "why authors did not consider alkanes but only FAs", authors answered "if we can do the attribution with FAs why increase analytical effort and use alkanes in addition?" This answer is surprising, because in Schindler et al 2012, authors analyzed the same set of samples for their _13Ctot, _ 15N, _ 13Corg contents and C/N ratio, with the same rationale than in the present work. And they successfully answered to the initial scientific question. Why did Authors spend time consuming and expensive cost analysis, if isotopic analyses on bulk sediments,(which are less expensive analysis (in time and cost)) were shown to be sufficient. Indeed, long chain n-alkanes are more reliable than FAs concerning terrestrial sources attribution in sediments.

Please see our statement at the beginning of this reply and also our changed formulations in the introduction.

Reply to Anonymous Referee #2

General comments

The paper deals with a sediment-fingerprinting tool that uses the 13C signature in organic biomarkers (FAs) allowing sediment source identification via an analytical solution of end-members along a mixing line. This paper presents a simple, but clear and well-developed case (including many previously missed caveats, e.g. use of long chain FAs, checking for tracer conservativeness and use of FA concentration rather %C to assess soil sources). The paper is very well written and I have no major comments and some specific remarks are listed below.

Thanks a lot for this positive comment which is very encouraging.

However, I disagree with the statements made in the abstract (Page 14246, line 7-10) and in the conclusions (page 14260, line 10-15). The later statement is clearly driven by the very simple case that was investigated. In reality most catchments are much more complex, as mentioned by the authors in the very first sentence of the abstract. According to me this has to be reformulated. I do

not see an analytical constraint (data quality analyses time) to analyze more FAs. Furthermore

new Bayesian mixing models (mixSIAR) have now many additional features (e.g. mixed and random effect, concentration dependency, etc.) to allow reliable distribution of estimates of sediment source proportions for complex landscapes (i.e. with>3 land uses), different sediments samples (event, vs. integrated) and samples taken at sub-catchment scale. Hence also aim iii) on page 14248 can only be achieved in this simple case and cannot be generalized for other, likely more complex cases.

In our statement to use the least possible data complexity we did not want to constrain analytics to FAs but generally would like to suggest not adding more tracers (and thus more complexity) to the approach than necessary. We agree that Bayesian mixing models seem to have many advantages in complex situations. Here we argue that with no significant differences in tracer signals between the two agricultural sources grassland and arable land, Bayesian mixing modelling would also not give a clear separation between the sources. E.g., just the mere use of a complex model with mixed and random effects and/or concentration dependency will not help to reduce the uncertainty originating out of the non-significant different tracer signatures between these two sources. Of course the modelling would be an advantage in case of significant differences between tracer signals and if an algebraic approach would not result in a (unique) solution. We have reformulated the parts regarding the modelling throughout the paper to be more precise.

Specific comments

The title is too general. Please make it more specific towards the case you studied.

We tried to be short and concise with our title but could, of course, be more specific. Our suggestion would be: "Quantitative sediment source attribution with compound specific isotope analysis in a C3 plant dominated catchment (Central Switzerland)"

Also what is CSIA? This is not clear here yet.

Sorry, abbreviation will not be used in the title anymore

Further be uniform sometimes you use "CSIA" vs. "CSSI". I think it should be "CSSIA".

CSIA (Compound Specific Isotope Analysis) is an established term in the isotope community and refers to stable isotope analysis only (e.g., not to compound specific radiocarbon analysis). Even though the reviewer is correct, that CSSIA would be a suitable abbreviation we would rather not introduce a new term, since the CSIA abbreviation is well established in the research community. If we talk about the isotopic signatures themselves, not about the analytics, the use of the term CSSI (compound specific stable isotopes) is suitable otherwise sentence structures and meaning does not make sense. Since we clearly defined our abbreviations when we first used them (with the exception of using CSIA in the title, sorry), we do not see any fault here.

Page 14247, line 8-13 is unclear. Please consider revising.

We revised the whole paragraph, please see line 41ff.

Page 14247, line 25. It is assumed that plant species have different 13C FA signals, but this is far from proven, although it is the basic (black box) assumption of the method used here. Please revise the sentence.

Yes, true. We changed the sentence to: "Although all plants produce the same FAs, the carbon stable isotopic signature ($\delta^{13}C$) of those biomarkers have been discussed to be different not only between aquatic compared to terrestrial organisms but also between different taxa of terrestrial C3 plants such as angiosperms and gymnosperms, trees versus herbs or for plant species adapting to environmental stress (Tolosa et al., 2013;Pedentchouk et al., 2008;Chikaraishi and Naraoka, 2007). The specific $\delta^{13}C$ signature of biomarkers is assumed to be preserved during degradation and transport (Hughen et al., 2004;Gibbs, 2008)."

Page 14248, line 19-21. Can you please indicate much better in the results and discussion and maybe the abstract where you show CSSIA signature preservations, hence that the tracers you have used are indeed clearly conservative, which is a crucial assumption in the method.

Our assumption is, that with a relatively small catchment and low number of possible sources, a fractionation of the CSSI of fatty acids would show in a deviation from the mixing line. This

is especially true when we solve for sediment source contribution at site A with one tracer only, but solving the equations with two different tracers (C:26 and C28 FAs).

We describe our approach in in paragraph 2.7 (line 220) : ”Deviation of CSSI of SS from the mixing line should not be greater than the procedural error or the measurement precision otherwise contribution of additional sources and/or isotope fractionation during degradation cannot be excluded.“ and also in the results (beginning of chapter 3.2.).

We did not include this in the abstract, because we feel this would be to complex and go beyond the short summary we can give in the abstract.

Page 14250. Maybe a criticism is the rather poor number of replicated samples of the sources. At least the authors could add of these replicates represent composite samples from 3 fields, which I hope it was otherwise this strongly reduces the statistical power of the investigation.

Sorry that we were not explicit enough on this. We added to chapter 2.3. the number of sample repetitions (line 138ff). and also calculation of the standard deviations chapter 2.8. line 241ff.

Page 14253, line 13. Explain “SS”.

done

Page 14254, Line 5-6. Please make clear if you refer to the 0.5 per mill for procedural error or to the FA-specific errors given on page 14253, line 8? Page 14255, line 23-26. Why were the FA-specific errors not considered here?

We decided to use the measurement uncertainty of 0.5 permil, due to the fact that the FA-specific error (procedural error) for C14:0FA (0.13 permil) and C28:0 FA (0.26 permil) were even smaller than the measurement uncertainty. In case of C26:0 FA (0.84 permil) the smaller value of the measurement uncertainty is tightening our requirements to the SS.

We added (chapter 2.7, line 222): “For unmixing of suspended sediment signature we decided to use the measurement uncertainty of 0.5‰ rather than the FA specific procedural error because the latter was even smaller for C14:0 FA and C28:0 FA. In case of the C26:0 FA a smaller value of the measurement uncertainty is tightening our requirements in respect to the sediment source attribution to the SS (e.g., the even larger error of 0.84 ‰ would allow a larger correction to the mixing line than we actually needed to do).”

Can you better explain how the

"forcing to mixing line" was carried out, i.e. the algebraic solution.

An explanation was added: "In case deviations from the mixing line occur that lie within this uncertainty of 0.5‰, we consider it valid to correct the measured isotope signals. The corrected value corresponds to the value at the intersect of the mixing line and a normal through the measured value."

Page 14256, line 6. Replace "bulking" by "averaging"?

Yes, correct, we change it.

Page 14256, Line 21-21. In your simple case (especially site A) Bayesian statistics would not at more info. But I would not generalize it (see comments) above and I would simply remove that sentence. I feel free to add I am not convinced the authors are aware of al recent developments and capacities (isotopic) Bayesian mixing models. The literature is plenty, but don't see any (recent) reference appairing.

Well, we quoted Smith and Blake, 2014 and Cooper et al., 2015a, who used Bayesion mixing modelling. But yes, we agree and deleted the sentence.

Page 14257, Line 12. Indicate this is the 13C signal in FAs

done

Quantitative sediment source attribution with compound specific isotope analysis in a C3 plant dominated catchment (Central Switzerland)

~~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~~ isotope~~s~~ 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,~~ (i~~i~~) ~~to use~~ using compound content (in our case long chain fatty acid (FA)) rather than soil organic matter content when converting isotopic signature to soil contribution and (ii~~i~~) ~~to~~ restricting evaluation to the long-chain FAs (~~C22~~C24:0 to C30:0) 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 connectivity of sediment source areas within the river changes between~~during~~ base and high flow conditions~~ connectivity of sediment source areas with the river change.changes~~. Due to t~~T~~he low data complexity (2-3 sources and 2 tracers) ~~helped to assess and avoid errors~~uncertainty which might ~~refraining~~arise from undetected source attribution and/or CSSI signature degradation, which might occur in ~~that are often obscured by~~ complex, large scale studies, is low. 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 a~~n~~ European perspective, sediment pollution has been identified as one of the most relevant pressures to water bodies which ~~will~~ impede~~d~~ to achieve the aims of the water framework directive by the year 2015 (Borja et al., 2006). Restoration of rivers from sediment impairment (such as clogging of river bed, eutrophication of waters, direct harmful effects of sediments on the biota and destruction of river infrastructure) and adapted management

strategies can only be efficient, if origin of sediment loads, contribution of sources and their connection to different land uses and management strategies are known. Geochemical ~~fingerprinting~~ (e.g., the use of elemental composition of source soils and sediments to track sediment origin) or isotopic fingerprinting has been used to discriminate between sources of sediments. However, the successful discrimination between different sediment sources was often restricted to certain catchment settings such as a change in geology or a shift from C3 to C4 dominated vegetation ~~or vice versa~~. ~~and was successful in discriminating between subsoil and surface soils but the technique is limited in providing significant differences between sources of different land use types and vegetation cover in complex landscapes.~~ . ~~If tracer signatures failed to be significantly different between sources, discriminant function analysis has been used in past studies to determine ifwhich the set of variables used would be would be most effective in predicting category (source) membership signatures (called category membership; . This set of tTracer signatures being classified as most suitable for fingerprinting were then used for sediment source attribution.~~

~~A new technique, u~~Using the compound specific stable isotope (CSSI) signatures of inherent soil organic biomarkers, can potentially discriminate and apportion the source soil contribution from different land-uses in order to reinforce the effectiveness of soil conservation measures (Gibbs, 2008;Blake et al., 2012;Guzman et al., 2013;Hancock and Revill, 2013;Ponton et al., 2014;Cooper et al., 2015a). The compound specific ~~stable~~ isotope analysis (CSIA) measures the $\delta^{13}C$ or $\delta^{2}H$ isotope signature of specific organic compounds associated with the organic matter bound to the soil/sediment. ~~Because of their polar nature, FAs are easily leached from the plant or the decaying plant material and become tightly bound to soil particles. Although all plants produce the same FAs, however the carbon stable~~

~~isotopic signature (δ^{13}C) of those biomarkers is different for each plant species (Chikaraishi and Naraoka, 2007;Pedentchouk et al., 2008;Tolosa et al., 2013)~~ In contrast to using the concentration of biomarkers as sediment tracers, the specific δ^{13}C signature of biomarkers is ~~and~~ assumed to be preserved during degradation and transport (Marseille et al., 1999;Hughen et al., 2004;Wiesenberg et al., 2004;Drenzek et al., 2007;Gibbs, 2008). As such, t~~T~~he CSIA method has already been successfully applied to link organic matter of sediments in estuarine or lake deposits to differentiate qualitatively between sources from algae, bacteria, zooplankton and higher plants and thus from terrestrial and aquatic sources (Galy et al., 2011;Tolosa et al., 2013;Fang et al., 2014;Ponton et al., 2014). In quantitative sediment attribution approaches~~,~~ the precision of the method was ~~impeded~~ constrained by the non-significant differences in the isotope signals between the different sources (Gibbs, 2008;Blake et al., 2012), especially if organic matter in sediment sources was dominated by C3 plant vegetation (Blake et al., 2012;Cooper et al., 2015a). The difficulty to differ sediment sources from soils from C3 vegetation land cover by CSIA of δ^{13}C in biomarkers ~~The latter~~ implied ~~a restriction to~~ (i) a restriction to ~~differ between~~ sources with vegetation shifts from C3 plants to the warm-climate C4 grasses, which are considerably higher in δ^{13}C values (Ficken et al., 2002;Quenea et al., 2006;Gibbs, 2008;Hancock and Revill, 2013;Cooper et al., 2015a), (ii) ~~to~~ achieving~~e~~ more effective discrimination by including information on δ^{2}H of *n*-alkanes (Cooper et al., 2015a) or (iii) ~~to include~~including geochemical mineral tracers for the fingerprinting (Blake et al., 2012) which is useful with obvious shifts in geologic bedrock of the soils. The above approaches restrict the application of ~~FAs~~ biomarkers as sediment tracers either to specific landscape settings (shift in geologic bedrock, shift from C3 to C4 plant cultivation) and/or complicate the analytical procedures (additional analysis of complex

geochemical patterns or additional laborious analytical investigations on CSIA of biomarkers).

In this study, we used the $\delta^{13}C$ of fatty acids (FAs) to discriminate between soil sources of different land use types (forest, pasture and arable land). All plants produce the same FAs, however the carbon stable isotopic signature ($\delta^{13}C$) of those biomarkers have been discussed to be different not only between aquatic compared to terrestrial organisms but also between different taxa of terrestrial C3 plants such as angiosperms and gymnosperms, trees versus herbs or for plant species adapting to environmental stress (Chikaraishi and Naraoka, 2007;Pedentchouk et al., 2008;Tolosa et al., 2013). Because of their polar nature, FAs are easily leached from the plant or the decaying plant material and become tightly bound to soil particles. If source soils from differing land cover fail to have significantly different CSSI signatures this might be due to one or a combination of the following reasons: measurement imprecision of CSIA (procedural error), soil heterogeneity and low sample numbers and/or changes in land use (former forests might now be grasslands or grasslands might now be arable soils. As such ~~todays~~today's source soils might have mixed signals).

In contrast to previous studies, we chose a relatively simple setting with three land use types only to evaluate whether or not sediment origin from soils with C3 plant cover only can be differentiated by CSSI signature. ~~Forests in the area are on calcareous bedrock with a step geomorphology which makes a previous land use as grassland or arable soil very unlikely.~~ ~~CSSI signature~~~~Furthermore, this~~ The constrained setting will allow evaluating the validity of the assumption that CSSI signature is preserved during degradation and transport. Further,~~We were able to validate our~~ results may be verified against the previous study of Schindler Wildhaber (2012) attributing sediment sources with bulk isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$)

in the same study area. ~~a landscape setting with a~~The latter was possible due to a shift from calcareous to siliceous bedrock that coincide~~dnt~~ with a shift in land cover. Forests in the study area are on calcareous bedrock with a pronounced topography which makes a previous land use as grassland or arable soil very unlikely.

Our aim was sediment source attribution from three different land use types within the Enziwigger catchment (Canton Lucerne, Switzerland) in (i) evaluati~~o~~ng differences of $\delta^{13}C$ signature in fatty acids~~CSSI signatures~~ of soil samples from possible sediment source areas dominated by C3 vegetation land use types and~~,~~ (ii) comparing the CSSI source signatures to tracer signals of suspended sediments in the river captured within a two year study (2009-2010)~~ and (iii) reducing method uncertainty in reducing the complexity of the unmixing procedure~~.

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^2. 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. ~~ure~~ 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, 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 requires to identify ~~means we cannot separate them from the~~ riverine FA production not connected to sediment transport (see below).

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 soil sample represents a composite sample of three cores. In addition, each site was sampled~~, 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^3) 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 48h, 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 seconds at a frequency of 24/s.

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).

2.5 Lipid extraction and preparation

Soil samples (11-21 g) and suspended sediments ~~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 $\delta^{13}C$ 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°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°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°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°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% BF_3 in MeOH was added. Methylation reaction of free FAs to fatty acid methyl

esters (FAMEs) took then place at 60°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°C. Samples were extracted in three different extraction batches. 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.

~~To monitor the quality of lipid extraction and analysis 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, Walthalm, MA 02451, USA). GC oven temperature started at 50°C and was increased to 150°C at a speed of 10°C/min, hold for 1 min, increased to 300°C at a speed of 4°C/min and hold for 63 min. The carrier gas helium was set to a constant flow of 1 ml/min. 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. For error estimation triplicate analysis was done for three samples from the same sample bag (see above). Standard deviation was < 5% for all FA concentration (see 2.7.).

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. ~~Then it~~Temperature was hold for 2 min and increased to 300°C at a speed of 4°C/min and hold for 35 min. The carrier gas helium was set to a constant flow of 1.2 ml/min. 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 $\delta^{13}C$ values have been corrected for the additional carbon atom introduced during methylation and had an analytical uncertainty lower than ±0.5‰.

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 of the ~~(~~source soils~~)~~ in multiplicities as procedural controls to estimate the reproducibility within the analysis procedure (from taking the soil sample out of the sample bag, via the lipid extraction, methylation, identification and quantification of FAs up to the final determination of the CSSI) ~~and~~as well as the heterogeneity in one sample bag. We analysed ~~three times~~ a sample out of the same sample bag three times including lipid extraction (pasture, s~~S~~ite C) and resulted in an overall procedural standard deviation of 0.13, 0.84 and 0.26 ~~permil~~ ‰ $\delta^{13}C$ 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, supporting information). To establish mixing lines for sediment source attribution we calculated mean values of source areas (Figs.~~ure~~ 2-3). Deviation of CSSI of suspended sediments ~~SS~~ from the mixing line should not be greater than the procedural error or the measurement precision otherwise contribution of additional sources and/or isotope fractionation during degradation cannot be excluded. For unmixing of suspended sediment signature we decided to use the measurement uncertainty of ~~(~~0.5‰~~)~~ rather than the FA –specific procedural error because the latter ~~is~~ was even smaller for C14:0 FA and C28:0 FA~~ even smaller~~. ~~And i~~In case of the C26:0 FA a smaller value of the measurement uncertainty is tightening our requirements in respect to the sediment source attribution to the SS (e.g., the even larger error of 0.84 ‰ would allow a larger correction to the mixing line than we actually needed to do).

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., 2015b) 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 distributions, rather simply the range of values that might be plausible given the geometry of the system.

In this study, we have a very limited number of sources (2 for site A and three for site B and C). ~~These have the following composition.~~ For ~~S~~site A forest as well as pasture value consists of 3 sample areas~~, same is true for Pasture at Site A~~. Since site B includes sub-catchment A and B, and catchment C includes A, B and C, these values include 3 forest/pasture areas from each site A and B~~,~~ and C respectively. Arable land value consists of 3 areas for s~~S~~ite B and 6 for s~~S~~ite C (3 from s~~S~~ite B plus 3 from s~~S~~ite C). The averaged agricultural land value at s~~S~~ite B consists of 6 pasture areas (A, B) and 3 arable land areas (B), and at s~~C~~ite C, 9 pasture areas (A, B, C) and 6 arable land areas (B, C). Standard deviations of the averaged values ~~you find~~are given in Table S1. Due to the linear arrangement of the problem we prefer the calculation of a unique algebraic solution, however, including the uncertainty ranges resulting of ~~the procedural error~~measurement uncertainty.

In case deviations from the mixing line occur that lie within the measurement uncertainty ~~associated with the procedural error~~ of 0.5‰, we consider it valid to correct the measured isotope signals to the mixing line. The corrected value corresponds to the value at the intersect of the mixing line and a normal through the measured value. We applied IsoSource with a tolerance value equivalent to the measurement uncertainty ~~Only~~ only if a~~n~~ unique algebraic solution was not possible~~,~~ due to the non-significant differences between the sources ~~we applied IsoSource with a tolerance value equivalent to the procedural error~~.

2.9 Weighting sediment source attribution according to FA content

The CSIA ~~method~~ rather traces the FAs which bind to the soil particles as part of the organic matter than the mineral soil sediments itself. Therefore, results need to be adjusted to account for the different amounts of each FA in each of the soil sources and to convert signature~~s~~ contribution into soil contribution to suspended sediments:

$$\%Soilsource_n = \frac{(P_n/FA_n)}{\sum_n (P_n/FA_n)} \times 100$$

Where P_n is the proportion ~~for~~ of soil *n* resulting from the unmixing of FA signatures, and FA_n is the sum of concentrations of fatty acids used for discrimination in the soil.

3 Results and Discussion

3.1 CSSI signatures of terrestrial soil sources

From all FAs analysed (even numbered from C14:0 to C30:0), the C18:0, C22:0, C26:0 and C28:0 FAs showed significant differences ~~(T-test)~~ between the sources forest and pasture soil as well as forest and arable soil (supporting information, Tables S1 and S2). The C26:0 and C28:0 FAs resulted in greatest differences with highest significances between forest and agricultural land use (Tables S1 and S2). For the difference between pasture and arable land, only the CSSIs of the C14:0 FA ~~was~~ were significantly different ($p < 0.043$). Thus, w~~W~~e found ~~five~~ four tracers to differentiate between sediment sources from forest and agricultural land use (pasture and arable land) but only one tracer (C14:0) to distinguish pasture and arable land sediment contribution. In our study, with a maximum of three different land use

types (forest, grassland and arable land), we should be able to separate the source attribution at all our sites with two tracers without the use of mixing models.

3.2 Unmixing of suspended sediment signatures

Following the theoretical concept of n tracers with n+1 sources, we only need one tracer for site A where sediments might origin from only two different land use types. However, using only one tracer, no mixing line can be established and deviations from mixing lines, either due to the influence of an additional source or due to degradation during transport, will not be recognized. The latter can be overcome due to the fact that several significantly different tracer signals should result in the same source attribution. This is the case if the suspended sediments (SS) plot exactly on the mixing line between the two different tracers. In general, whether or not using a mixing model, the isotopic values of the sediment mixture being evaluated must be within the isotopic values of the source endmembers (Phillips and Gregg, 2003). In our case, suspended sediments are not exactly on the mixing line between the two source soils (Fig.~~ure~~ 2), which resulted in differences of up to 15% for source attribution at site A using either the C26:0 or the C28:0 FA.

Since the deviation from the mixing line is within the uncertainty associated with the measurement precision ~~or the procedural error~~ of 0.5‰, we consider it valid to correct the measured isotope signals in forcing them on to the mixing line for sediment source apportionment~~ with a linear regression~~ (Fig.~~ure~~ 2). When using the stable isotope signals which were corrected by regression to the intersect value of the mixing line, sediment source attribution results in the same source attribution for both tracer applications (Table 1). The question whether the CSSI signature is preserved during degradation and transport cannot be answered ~~assuredly~~with absolute certainty. We observe a small but systematic deviation of

the SS signal from the mixing line (Fig. 2) which could be ~~Nevertheless, we cannot exclude~~due to a small contribution from an additional source and or a slight degradation of the signal during transport. Nevertheless, the effect is very small and lies within the magnitude of the ~~procedural error~~measurement uncertainty.

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, S2). 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.~~ure~~ 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 only produced by higher plants but by microorganisms and algae, mainly by aquatic algae (Lichtfouse et al., 1995;Huang et al., 1996;Hughen et al., 2004;Eglinton and Eglinton, 2008;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 (SS) 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 (C2~~2~~4:0 to C30:0).

Because of the non-significant differences between the CSSI signatures of long chain FAs of pasture and arable land (Fig. ~~ure~~ 3), we can solve ~~for~~ the sediment contribution at sites B and C ~~also~~ only ~~from~~ for two different sources ~~if we want to remain statistically firm~~: forest versus agricultural land (the latter ~~bulking~~ averaging 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~~ to 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 (Figure 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 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 (SS) 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 we would highly suggest constraining the concept of tracking terrestrial sediments to the long-chain FAs (C22:0 to C30:0).~~

Because ~~the~~ with CSIA ~~method~~ we trace~~s~~ ~~carbon~~ FAs rather than the soil itself, the results given by the unmixing of the $\delta^{13}C$ signals of FAs need to be adjusted to account for the different amounts of each of the soil sources. Following solutions in the 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~~ure~~ 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.

3.3 Apportionment of suspended sediment during high and base flow

Following the above sediment source attribution approach at site A during base flow, 30% and 70% of sediments were contributed from pastures and forests, respectively (Table 1). Downstream, at sites B and C, sediments from agricultural sources increase considerably during base flow (65% from agricultural sources and 35% from forests) reflecting the contribution from more intensively used arable land and pasture. At the two investigated high flow events, sediment sources varied considerably at site A (between 15 and 40% from pastures and between 60 and 85% from forests) and site B and C (contribution between 6 to 45% from agricultural land and 55 to 93% from forests), with sediment contribution from forests clearly being dominant during high flow events.

Our results are consistent with Schindler Wildhaber et al., (2012a) where sediment source attribution was achieved with bulk isotope signals (the latter was feasible due to the change in geology from calcareous bedrock under forest soils and siliceous bedrock under agricultural soils).

Results indicate that connectivity of sediment source areas with the river change from base to high flow regime. Management options to decrease sediment peaks during storm events should thus aim at adapted forest management (e.g. increasing soil and understorey vegetation). ~~even though the latter will be difficult due to extremely steep slopes and loosely~~

~~structured calcareous soils under forests in the Enziwigger catchment.~~ The dominance of forest soil sources to sediment contribution during high flow is an important and surprising result since typically agricultural areas are in the focus of soil conservation management. The larger forest contribution is likely conditioned by ~~even though the latter will be difficult due to~~ the extremely steep slopes and loosely structured calcareous soils under forests compared to the flat arable land on siliceous bedrock in the Enziwigger catchment.

Separation between the agricultural land use types pasture and arable soil with IsoSource pointed to the same direction as the unique algebraic solution regarding the high forest contributions during high flow (Table 1). The difference between the IsoSource results and our unique solutions regarding the forest contribution are between 3 and 15% at sites B and C. Sediment source attributions according to the 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;Alewell et al., 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, quantitative 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~~ found 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~~ short chain fatty acid. We recommend using long chain fatty acids (~~C22~~C24:0 to C30:0) only for sediment 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.~~Small scale studies with well-defined sediment sources and significant differences in CSSI signature may help to verify the suitability of the CSIA as a sediment fingerprint technique in fluvial systems.

Author contribution:

Christine Alewell: project idea, concept and initiative, data interpretation, manuscript writing

Katrin Meusburger: data evaluation, IsoSource modelling, manuscript writing

Axel Birkholz: method development, CSIA, data evaluation, manuscript writing

Yael Schindler Wildhaber: field study concept, sampling of suspended sediments, interpretation

Lionel Mabit: interpretation, manuscript writing

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Tables

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

		2 Tracer/2 Sources				2 Tracer/3 Sources (IsoSource)					
Site	Event	% 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 s~~S~~ite B a unique solution was possible.

Figure captions

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:

$\delta^{13}C$ 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, $\delta^{13}C$ were corrected to the mixing line ~~with linear regression~~. Error bars of SS display the ~~procedural~~ measurement error of 0.5 ‰.

Figure 3:

$\delta^{13}C$ 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~~ measurement error of 0.5 ‰.

Figure 4:

~~-~~FA concentration compared to % Corg at the source sites. The first letter gives the site notation (sites A, B, C) while the second letter ~~indicated~~ indicates the land use type (F = forest, P = pasture, A = arable land).