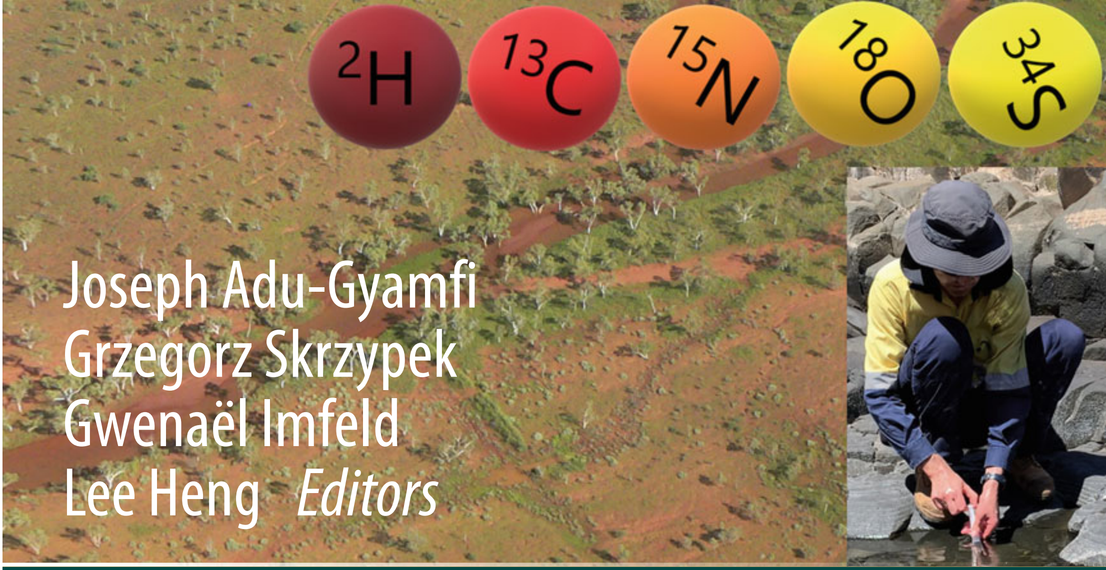

Joseph Adu-Gyamfi
Grzegorz Skrzypek
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Lee Heng *Editors*

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Applications of Multi-stable Isotopes

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Preface

Pollution originating from agriculture, domestic water waste, mining sites, sediment and soil erosion, and other sources has a direct negative impact on human health and water, food, and natural environment quality. Solutes such as nutrients, fertilizers, pesticides, or their by-products disperse through natural ecosystems contaminating streams, rivers, lakes, and groundwater aquifers. Pollutant concentrations frequently increase downstream, and many agroecosystems are contaminated by multiple pollution sources, but traditional techniques based on chemical concentrations do not allow evaluation of the relative contributions from different sources. Complementarily to conventional monitoring, more advanced multi-tracer approaches are required. Stable isotope compositions of nitrates $\delta(^{15}\mathrm{N})_{\mathrm{NO_3}}$, $\delta(^{18}\mathrm{O})_{\mathrm{NO_3}}$, water $\delta(^{2}\mathrm{H})_{\mathrm{H_2O}}$, $\delta(^{18}\mathrm{O})_{\mathrm{H_2O}}$, sulphates $\delta(^{34}\mathrm{S})_{\mathrm{SO_4}}$, $\delta(^{18}\mathrm{O})_{\mathrm{SO_4}}$, phosphates $\delta(^{18}\mathrm{O})_{\mathrm{PO_4}}$, and carbon $\delta(^{13}\mathrm{C}_{\mathrm{POM\ or\ DIC}})$ combined with advanced hydrochemical methods and models over the last couple of decades have become important methods in forensic investigations of water quality. However, major knowledge gaps in pollution studies in agroecosystems still exist, including the source characterization and separation of mixing from isotope fractionation and equilibration. Therefore, more data, research, and integration of approaches are required. There is also a need for method standardization and the formulation of a scientific *toolbox* that assembles available sampling and analytical techniques useful for identifying sources and transport of agropollutants for better understanding, managing, and improving soil, crop, surface, and groundwater quality in agroecosystems.

The Soil and Water Management and Crop Nutrition (SWMCN) Subprogramme of the Joint Food and Agriculture Organization (FAO)/International Atomic Energy Agency (IAEA) Centre of Nuclear Techniques in Food and Agriculture, through a Coordinated Research Project (CRP) in partnership with national and international research institutes, developed and evaluated a set of analytical techniques, i.e. the *toolbox*. This *toolbox* integrates multiple isotope tracers that provide information on the origins and pathways of multiple pollutants through agroecosystems, thereby providing more accurate guidance on mitigations. The objective of this book is to present protocols, methodologies, and standard operating procedures (SOPs) used for the identification of sources, transport, and fate of agrocontaminants and illustrate them with several case studies of successful applications. However, land management strategies to address and control the transport of pollutants from soil to water bodies remain the shared responsibility of farm and aquaculture operators, agrochemical manufacturers, and policymakers in food and agriculture, as well as the mining sectors. This book is structured into eight chapters covering (i) an overview of the book's content, (ii) guidelines for designing water sampling programmes, (iii) the use of mixing models applicable to tracers for water pollution studies, (iv) compound-specific isotope analyses to investigate pesticide degradation in agricultural catchments, (v) the use of stable oxygen isotope composition of phosphate to investigate phosphorous in soil-plant continuum, (vi) the use of stable sulphur isotopes to disentangle agropollutants from other contaminants, (vii) nuclear tools used in sediment source apportionment, and (viii) the conclusions and perspectives forward. The book offers up-to-date information, and we hope it will be a great source of information and inspiration for students, researchers, and policymakers.

The SWMCN subprogramme and the editors thank all the contributors involved in the preparation of this publication.

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Contents

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Chapter 1

Introduction: Stable Isotope Tracers Used for the Identification of Contaminants in Agro-Ecosystems

G. Skrzypek, J. Adu-Gyamfi, G. Imfeld, and L. Heng

Abstract This chapter provides an overview of the stable isotope tracers used in agro-contaminant studies, summarizing essential information about each tracer, including the range of values, sample collection and preparation, and the most common applications. The chapter provides an overview of the book's concept and contents and refers readers to the chapters of interest.

Sources of pollution in agro-ecosystems are usually difficult to identify using pollutant concentrations alone. Most of these sources are not attributed to a specific location (non-point) and are typically in solution (non-particulate). Therefore, compound concentrations can inform about the level of pollution (low or high), temporal and spatial dispersal but cannot directly confirm the actual source and transformation of the pollution. To unambiguously identify sources of pollution, a more advanced approach is needed, e.g., using tracers that carry unique fingerprints of each potential source. Frequently stable isotope compositions of elements forming the chemical compound in pollution are used as environmental tracers. In conjunction with the major ion ratios and stable hydrogen and oxygen isotope compositions of water

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molecules, these isotope tracers can be successfully used to identify sources of pollutants found in surface and groundwaters (Kendall 1998; Marshall et al. 2008). The application of mass balance calculations using isotope tracers often allows for not only qualitative but also quantitative estimations of the relative contributions from different sources of pollution and the discrimination of agricultural inputs from other contaminants (Kendall 1998). The stable isotope signatures and ion ratios also provide information that is independent of solute concentrations, thereby disentangling the water budget from the pollution budget. An accurate identification of sources and transport of pollutants from soil to water in agro-ecosystems will help develop appropriate soil and water management practices to minimize agropollutants to surface and groundwaters.

Two primary challenges need to be addressed to improve our ability to monitor and quantify sources and transport of pollutants in agro-ecosystems using stable isotope tracers. The first lies in the reliable and routine use of stable isotope methods to identify pollutants and distinguish mixing from stable isotope fractionation. The second challenge lies in the comprehensive implementation of a multi-tracer approach relying on two or more elements and their stable isotope compositions to characterize and quantify sources and reactive transport of solutes in agro-ecosystems. In the present book, we address these two challenges by focusing on the stable H, C, N, O and S isotope compositions in several chemical compounds to trace and monitor sources and transport of macronutrients and micro-contaminants in soil and waters under different land uses and hydro-climatic conditions. Stable isotope tracers have great potential to improve the understanding, management and protection of surface and groundwater and to help in the qualitative and quantitative prediction of water resources. The six chapters included in this book present a critical overview of the stable isotope tracer methods forming a hydrochemical toolbox to be applied in the broad range of agro-contaminant studies. We thus anticipate that this book will stimulate researchers and managers to further develop and use hydrochemical and stable isotope techniques worldwide, complementarily to conventional monitoring and mass balance approaches.

The most commonly used analyses of the stable isotope composition are those of the solutes in water [$\delta(^{15}\mathrm{N})_{\mathrm{NO3}}$, $\delta(^{18}\mathrm{O})_{\mathrm{NO3}}$, $\delta(^{34}\mathrm{S})_{\mathrm{SO4}}$, $\delta(^{18}\mathrm{O})_{\mathrm{SO4}}$, $\delta(^{13}\mathrm{C})_{\mathrm{DIC}}$, $\delta(^{18}\mathrm{O})_{\mathrm{PO4}}$, $\delta(^{13}\mathrm{C})_{\mathrm{pesticide}}$, $\delta(^{15}\mathrm{N})_{\mathrm{pesticide}}$], the particulate organic matter [$\delta(^{13}\mathrm{C})_{\mathrm{POM}}$, and $\delta(^{15}\mathrm{N})_{\mathrm{POM}}$], together with water molecules [$\delta(^{2}\mathrm{H})_{\mathrm{H2O}}$ and $\delta(^{18}\mathrm{O})_{\mathrm{H2O}}$], as well as chemical ion concentrations, and the major ion ratios (e.g., $\mathrm{Na^+/Ca^{2+}}$, $\mathrm{Cl^-/SO_4{}^{2-}}$, $\mathrm{Ca^{2+}/SO_4{}^{2-}}$). All these analyses are particularly useful for tracing the dynamics of pollution contributions from different sources, usually in reference to local baselines and the original signatures of the contributing pollution sources. The major advantages of stable isotope methods are their relatively low cost and high effectiveness, even when used in areas lacking long-term monitoring or a comprehensive sampling network.

The stable hydrogen, carbon, nitrogen, oxygen and sulfur isotope compositions (HCNOS) of various compounds can be used to study agricultural pollution in surface and groundwaters by applying mass balance calculations and other models to assess the progress of dispersal or decomposition (Chap. 3). Different stable isotope tracers usually carry different and complementary information about pollution sources, water flows and mixing, as well as about pollutant chemical transformations in the aquatic environment (Chaps. 4–7). Various tracers can be combined to obtain a more unambiguous characterization of pollution sources and to disentangle water budgets from solute budgets, or they can be used independently to address different environmental questions (e.g., Szynkiewicz et al. 2015; Elsner and Imfeld 2016; Dogramaci et al. 2017; Pfahler et al. 2022). This brief overview provides a list of the commonly used stable isotope tracers discussed in this book, along with their major applications, the general ranges of the δ-values observed in nature and the expected analytical uncertainty (Coplen et al. 2002a, b). The analytical uncertainty, along with the natural variability of the δ-values in each source, needs to be considered for the correct solution of the mass balance mixing model equations. One critical consideration is whether the δ-values in the sources potentially contributing to the analysed mixture are significantly different from each other. If the δ-values of different sources are similar, models cannot be solved with high confidence to distinguish sources. On the other hand, transformation processes may be traced over space and time if δ-values in the sources are similar.

The list of tracers presented below has been arranged considering their relevance to studies of agricultural pollution in terrestrial environments. The meaning of the categories is as follows:

- *analysed in*—medium in which the stable isotope compositions can be analysed.
- *range of values*—an approximate typical range of the δ-values frequently observed in natural terrestrial environments.
- *uncertainty*—the typical uncertainty of the stable isotope analysis given as one standard deviation; it varies, respectively to the analytical method used.
- *applications*—primary applications of the tracer.
- *zero value*—the reference point for the scale characterized by the δ-value equal $0\,‰$.

For the full range of variation in stable isotope compositions in all environments and compounds reported around the world, see the summary by Coplen et al. (2002a, b).

$\delta(^{2}H)_{H2O}$ and $\delta(^{18}O)_{H2O}$—stable hydrogen and oxygen isotope compositions of water

analysed in: surface, ground, precipitation and soil water

range of values: $\delta(^{2}\mathrm{H}) - 150\,‰$ to $+ 50\,‰$ and $\delta(^{18}\mathrm{O}) - 20\,‰$ to $+ 10\,‰$

uncertainty: $\delta(^{2}\mathrm{H})$ ~ 0.5–1.0 ‰, $\delta(^{18}\mathrm{O})$ ~ 0.05–0.10 ‰

applications: used for tracing sources, mixing models, assessing evaporative losses of water

zero value: the stable isotope compositions of the Vienna Standard Mean Ocean Water (VSMOW) are accepted as a zero-reference point ($0\,‰$) for $\delta(^{2}\mathrm{H})$ and $\delta(^{18}\mathrm{O})$

on the VSMOW2-SLAP2 scale. Therefore, $\delta(^{2}H)$ and $\delta(^{18}O)$ values close to zero are typical for seawater and moderately evaporated freshwater.

$\delta(^{15}N)_{NO3}$ and $\delta(^{18}O)_{NO3}$—stable nitrogen and oxygen isotope compositions of nitrates

analysed in: NO_3^- ions dissolved in water or NO_3^- in solid substances, e.g., fertilizers, salt precipitates

range of values: $\delta(^{15}N)$ -10 ‰ to + 25 ‰ and $\delta(^{18}O)$ -15 ‰ to + 80 ‰

uncertainty: $\delta(^{15}N)$ ~ 0.3–0.5 ‰ and $\delta(^{18}O)$ ~ 0.3–0.5 ‰

applications: usually used to distinguish N-input sources of surface and groundwater pollution and to assess N-contributions from precipitation, natural plant decomposition, fertilizers, manure and human wastewater; significant fractionation can be caused by the nitrification–denitrification cycle influencing stable isotope composition and substrate concentrations

zero value: the stable isotope composition of atmospheric nitrogen is accepted as a zero-reference point for the nitrogen air scale [$\delta(^{15}N) = 0$ ‰]. The $\delta^{15}N$ value of atmospheric N_2 is constant around the world. The $\delta(^{18}O)$ is usually reported on the VSMOW2-SLAP2 scale, which is the same as water.

$\delta(^{34}S)_{SO4}$, $\delta(^{18}O)_{SO4}$—stable sulfur and oxygen isotope compositions of sulfates

analysed in: SO_4^{2-} ion dissolved in water or SO_4^{2-} in solid substances, e.g., fertilizers, salt precipitates; frequently, for reference, $\delta(^{34}S)_{SO4}$ is analysed as well in sulfides and various fractions in soil

range of values: $\delta(^{34}S) - 25$ ‰ to + 40 ‰ and $\delta(^{18}O) - 20$ ‰ to 30 ‰

uncertainty: $\delta(^{34}S)$ ~ 0.2–0.4 ‰, $\delta(^{18}O)$ ~ 0.2–0.4 ‰

applications: used to assess inputs from fertilizers and wastewater and to distinguish them from acid rock and mine drainage and atmospheric pollution

zero value: the stable isotope composition of Vienna Canyon Diablo Troilite (VCDT), a meteorite mineral, is accepted as a zero reference for the sulfur scale [$\delta(^{34}S) = 0$ ‰]. The $\delta^{18}O$ is usually reported on the VSMOW2-SLAP2 scale.

$\delta(^{13}C)_{POM}$ and $\delta(^{15}N)_{POM}$—stable carbon and nitrogen isotope compositions of particulate organic matter suspended in water (POM)

analysed in: particulate organic matter as a suspension in water or sediments, frequently for reference also analysed in plant material, and soil, usually POM is separated on fibreglass filters by water filtration

range of values: $\delta(^{13}C) - 30$ ‰ to $- 10$ ‰ and $\delta(^{15}N) - 5$ ‰ to + 20 ‰

uncertainty: $\delta(^{13}C)$ ~ 0.10 ‰ and $\delta(^{15}N)$ ~ 0.10 ‰

applications: primarily used to estimate C-inputs from different vegetation types (especially agricultural crops) and soil erosion to surface water and to distinguish terrestrial and aquatic C-sources; also frequently used to assess C3 to C4 plant input to soil or suspended particles

zero value: the stable isotope composition of Vienna Pee Dee Belemnite (VPDB), a carbonate marine fossil, is accepted as a zero-reference point for $\delta(^{13}C)$ stable isotope scale. Therefore, marine carbonates have $\delta(^{13}C)$ close to zero.

$\delta(^{18}O)_{PO4}$—stable oxygen isotope compositions of phosphate

analysed in: PO_4^{3-} dissolved in water, in plant material, soil, fertilizers, sediments, rocks, detergents, rivers and vegetation

range of values: + 6 to + 31 ‰

uncertainty: ~ 0.3–0.5 ‰

applications: tracing phosphate sources and phosphate turnaround time in soil and surface water to assess fertilizer leaching and turnover

zero value: as above for water.

$\delta(^{13}C)_{DIC}$—stable oxygen isotope compositions of dissolved inorganic carbon (DIC)

analysed in: water, usually as total inorganic dissolved carbon (the ratio between $CO_2/HCO_3^-/CO_3^{2-}$ in solution depends on pH)

range of values: − 20 ‰ to + 5 ‰

uncertainty: ~ 0.10–0.20 ‰

applications: primarily used to confirm decomposition of hydrocarbon pollution and methane oxidation and their contribution to DIC; also, to assess the contribution of aquatic organisms to diel cycles of DIC; to detect contributions from deep groundwater

zero value: as above for $\delta(^{13}C)_{POM}$.

$\delta(^{13}C)$ and $\delta(^{15}N)$—stable carbon and nitrogen isotope compositions of pesticide molecules

analysed in: water, soil, sediment and plants

range of values: − 35 ‰ to 0 ‰ for $\delta(^{13}C)$ and − 4 ‰ to + 4 ‰ for $\delta(^{15}N)$

uncertainty: ~ 0.5 ‰ for both $\delta(^{13}C)$ and $\delta(^{15}N)$

applications: primarily used to evaluate in situ degradation, without considering concentration data of parent compound or transformation products, quantify pollutant degradation and, in some cases, identify reaction pathways *zero value*: the stable isotope composition of Vienna Pee Dee Belemnite (VPDB), a carbonate marine fossil, is accepted as a zero-reference point for $\delta(^{13}\mathrm{C})$ stable isotope scale. The stable isotope composition of atmospheric nitrogen is accepted as a zero-reference point for the nitrogen air scale [$\delta(^{15}\mathrm{N}) = 0$ ‰]. The $\delta(^{15}\mathrm{N})$ value of atmospheric N_2 is constant around the world.

All stable isotope results in this book are presented in permille (‰) as 1000 isotope delta, on the stable isotope scale respective to the element (e.g., VPDB, VSMOW2-SLAP2, VCDT or AIR). The isotope delta (symbol δ) is defined by the isotope ratios of heavier (i) to lighter (j) isotope of an element (E) in substance (P) and isotope ratio of this element in an international standard (Std), defining the zero point for the international isotope scale, 0 ‰ (Eq. 1.1):

$$\delta\left(^{i/j}\mathrm{E}\right)_{P,\mathrm{Std}} = \frac{R\left(^{i/j}\mathrm{E}\right)_{P}}{R\left(^{i/j}\mathrm{E}\right)_{\mathrm{Std}}} - 1 \tag{1.1}$$

This full notation, for example, for stable sulfur isotope composition in sulfate, respectively to VCDT scale can be expressed as follows (Eq. 1.2):

$$\delta\left(^{34/32}\mathrm{S}\right)_{SO_4,\mathrm{VCDT}} = \frac{R\left(^{34/32}\mathrm{S}\right)_{\mathrm{SO_4}}}{R\left(^{34/32}\mathrm{S}\right)_{\mathrm{VCDT}}} - 1. \tag{1.2}$$

To avoid excessive repetition of common terms, we used a shorthand version of the full notation (Eq. 1.3):

$$\delta\left(^{34}\mathrm{S}\right)_{SO_4}. \tag{1.3}$$

The delta values are reported without corrections or adjustments, exactly as in the source publications. Readers should be aware that the isotope delta values assigned for international standards undergo occasional changes, and discrepancies may arise while pooling values published during different years. To warrant the reusability of published data, all new stable isotope results should be published following minimum requirements for publishing HCNOS stable isotope delta results guidelines (Skrzypek et al. 2022) and correct terminology and notations endorsed by the International Union of Pure and Applied Chemistry (Cohen et al. 2007; Brand et al., 2010; BIMP 2019). Each stable isotope data set should be accompanied by a description of (1) analytical procedure, (2) traceability, (3) data processing and (4) uncertainty evaluation.

Other Tools for Tracing Anthropogenic Contaminants

Other less frequently used tracers for tracing anthropogenic contaminants in water and soil are boron [$\delta(^{11}\mathrm{B})$ reflecting $^{11}\mathrm{B}/^{10}\mathrm{B}$ isotope ratio] and strontium [$\delta(^{87}\mathrm{Sr})$ reflecting $^{87}\mathrm{Sr}/^{86}\mathrm{Sr}$ isotope ratio]. The main anthropogenic source of boron (B) is sodium perborate ($NaBO_3$), which is used in laundry detergents and household cleaners; thus, boron is commonly found in household sewage. Sewage treatments generally do not remove boron or cause stable isotope fractionation (Barth 2000). Hence, $\delta(^{11}B)$ can be considered a conservative tracer for partitioning agriculture pollution (e.g., hog manure, cattle feedlot runoff and synthetic fertilizers) from household wastewater. The use of $\delta(^{11}B)$ coupled with $\delta(^{15}N)_{NO3}$ and $\delta(^{18}O)_{NO3}$ has proved to be an effective means of tracing agricultural nitrate sources to distinguish between two types of sewerage that were indistinguishable using $\delta(^{15}N)$ alone, including washing powders and animal/human manure (Guinoiseau et al. 2018; Kruk et al. 2020). Boron in household pollutants has values like boron minerals [$\delta(^{11}B) = 5$ to 13 ‰] significantly different from values observed in pristine groundwater (~ 30 ‰) or seawater (~ 39 ‰) (Vengosh et al. 1994; Vengosh 1998) and can be reflected in plant tissues (Chang et al. 2016).

In natural waters, the geochemistry of dissolved Sr is analogous to that of Ca. The Sr isotope fractionations in geochemical processes are considered negligible due to small differences in isotope masses (~ 1 %), and the $^{87}Sr/^{86}Sr$ ratios in natural systems mainly reflect geological age and the initial Rb/Sr ratio in the rocks (Gosselin et al. 2004). Thus, in groundwater, the $^{87}Sr/^{86}Sr$ ratio often reflects water–rock interaction time or the aquifer matrix if the groundwater contact time is long enough to attain equilibrium. Strontium isotope composition could be used for assessing salt budget and sources of salinity, particularly to partition fraction coming from rock erosion and precipitation and fertilizers (Hosono et al. 2007; Dogramaci and Skrzypek 2015).

Overall, pollutants may originate from various sources related to different types of agriculture activities, including cultivation, aquaculture, livestock and dairy farms and related food processing industries. Evaluating their respective contributions to soil, surface- and groundwater bodies remains, however, challenging. In this context, stable isotope tracers have untapped potential to quantify agro-contaminant sources, transport and transformation and distinguish them from other sources of contaminants, as presented below.

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Chapter 2
Conceptual Sampling Design for Tracing Agropollutants on a Catchment Scale

G. Skrzypek

Abstract This chapter provides guidelines for designing a sampling programme for tracing agricultural pollutants using stable isotope techniques at different catchment scales. This sampling template could be considered for the application of various tracers with respect to local needs and study objectives. The template is based on a series of overlapping mixing models that use 'triple sampling points' to identify major sources of pollution by estimating pollution budgets in different subcatchments along the water flow pathways. This framework can be further specified, and a more detailed sampling network can be developed after analysing the results obtained from the initial sampling. Step-by-step technical instructions on how to collect and preserve water samples for stable isotope analyses can be found in a separate document published by the International Atomic Energy Agency as a Standard Operating Procedure (Skrzypek in Sampling and isotope analysis of agricultural pollutants in water IAEA TECDOC SERIES. International Atomic Energy Agency, Vienna, pp. 2–21, 2018).

2.1 Conceptual Case Study—Sampling Design Outline

Water quality progressively deteriorates in a river supplying a lake, but the upstream catchment lacks hydrological infrastructure and monitoring points. The land use is diversified, and discharged pollution that impacts the lake water quality may originate from one or all activities conducted in the catchment, including dairy production, chicken farms, crops, fruit cultivation or mining. A sampling programme designed to identify pollution sources and their relative contributions to lake water quality requires optimised selection of sampling points across the whole catchment (see also the case study presented in Chap. 7).

The potential contributions from various sources of pollution are determined by dividing the catchment into a few subcatchments with expected different types and

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levels of pollution based on land use and infrastructure (Fig. 2.1). Each major tributary is sampled above and below potential sources of pollution and above and below the mouths of various order tributaries (mixing points). In this conceptual example, a total of 15 surface and 4 groundwater sampling points are used. The samples collected above mixing points (e.g., *b*2*, b*3) represent tracer concentrations and stable isotope compositions in the water outflowing from each subcatchment. The sample collected below the mixing point (e.g., *b*1) reflects a mean value that is proportional to the values of the samples (*b*2 and *b*3) collected above the mixing point and the volume of water arriving from each subcatchment.

Mean Signature of the Catchment

Concentrations of pollution at sampling point *a1* represent the mean value for the entire catchment. Some parts of catchments can be heavily polluted, whereas others can be pristine, and the pollution concentrations can be distinctive at different locations. Therefore, the mean concentrations will reflect the pollution loads and the volumes of water inflowing from different subcatchments that are characterised by different levels of pollution. The stable isotope compositions observed in water and water solutes reflect a mean value proportional to the stable isotope compositions and contributions from all significant pollution sources across the whole catchment. The

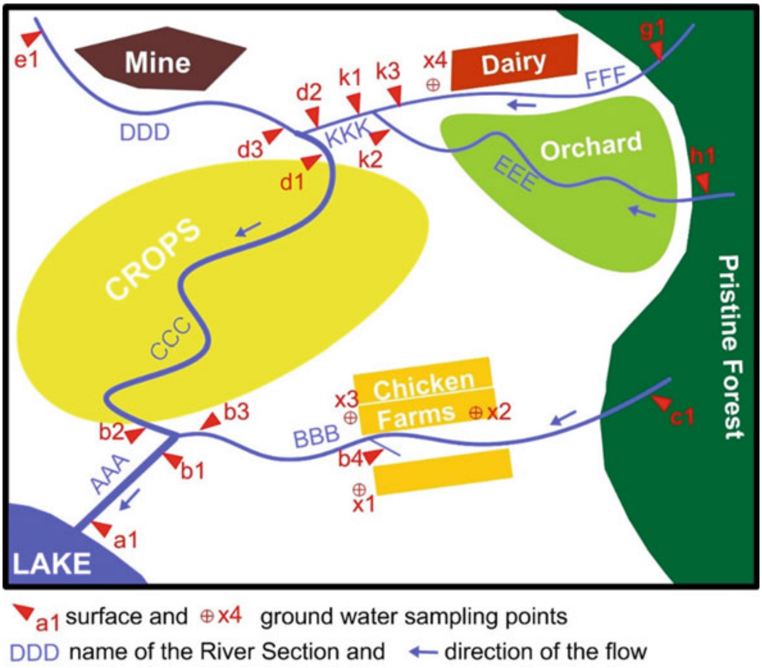

Fig. 2.1 Map of the conceptual sampling design on the catchment scale. This framework can serve as a template for selecting sampling points, although it requires accounting for local conditions, land use and research objectives

stable isotope compositions could also be further modified by secondary processes that cause stable isotope fractionation during the chemical transformation of pollution or its removal.

Baseline Background Values

All major ion concentrations and stable isotope compositions of chemical compounds that may occur in waters naturally or can be delivered with rainfall should be considered to understand the baseline values and to separate pollution originating from land use from natural concentrations and inputs from outside the catchment (Chap. 3). The baseline values can be established by sampling water in the section of tributaries above the expected sources of pollution (e.g., above a mine site, point $e1$) or from pristine areas (e.g., points $g1$, $h1$, $c1$, Fig. 2.1). Points $h1$ and $g1$ are located in the pristine area of a nature reserve and are not directly impacted by either agricultural or industrial activity. If these data are consistent with those obtained for other likely unpolluted points $e1$ and $c1$, they constitute a general baseline for this area and characterise the natural variability in solute concentrations and their stable isotope compositions.

The sampling of spring water or groundwater could also be considered. Additionally, the sampling of rainfall may provide useful information about the $\delta(^{2}\text{H})_{\text{H2O}}$ and $\delta(^{18}\text{O})_{\text{H2O}}$ values delivered to the study sites and for estimating evaporative losses. Rainwater chemical and stable isotope compositions can be useful for partitioning airborne pollution in regions where air pollution is a serious concern.

Subcatchment Division with Respect to Expected Pollution Sources

The studied catchment can be divided into a few subcatchments that separate sections of the major creek lines impacted by a specific type of pollution. These creek sections are named *AAA*, *BBB* to *FFF*. Surface water sampling points are labelled $a1$, $b1$ to $k2$; groundwater sampling points $x1$ to $x4$ (Fig. 2.1).

The river section *EEE* flows through the orchard, and if polluted, it can be expected to carry primarily fertilisers, pesticides and the other agrochemicals used in this area. The water quality of *EEE* can be analysed at the bottom of the subcatchment ($k2$) before it mixes with *FFF* to account for the pollutants originating from the orchard only. To understand the actual net input of pollution from the orchard to *EEE*, the quality of water entering the subcatchment should be considered and point $h1$ located in pristine area can be used to establish a baseline for water hydrochemistry entering this catchment. The difference between concentrations and stable isotope compositions between $h1$ and $k2$ reflects the added pollution loads from the orchard.

In contrast to *EEE*, the river section *FFF* may be impacted by pollution from two potential sources: the dairy factory and the orchard. Therefore, the difference between the results obtained for $k3$ and $g1$ (Section *FFF*) reflects the potential inputs from these two sources. The results for *FFF* can be further compared with the results for *EEE*. If the baseline values at $h1$ and $g1$ are not significantly different, then the difference between $k2$ and $k3$ could reflect the potential input from the dairy factory to *FFF*. The direct signature of potential pollution from the dairy factory can also be verified using groundwater (bore $x4$).

The proportional contribution of *FFF* and *EEE* to *KKK* and to lake pollution could be further calculated using mass balance calculations and the results for individual tracers from the triple sampling point $k3 + k2 = k1$. The concentrations and stable isotope compositions at sampling point $k1$ will reflect those at $k3$ and $k2$ with respect to the contribution from *FFF* and *EEE*. A simple verification procedure can be designed using the mass balance model (see calculation examples in Chap. 3) and the following equation (Eq. 2.1):

$$\delta_{k1} = x \times \delta_{k2} + y \times \delta_{k3}, \tag{2.1}$$

where δ_{k1}, δ_{k2} and δ_{k3} are stable isotope compositions of analysed samples collected at $k1$, $k2$ and $k3$. Assuming contributions from only two water sources, the proportional contributions x and y will equal 1 (Eq. 2.2).

$$x + y = 1. \tag{2.2}$$

Solving these simultaneous equations, we can calculate the relative contributions x and y that reflect the inputs from *EEE* and *FFF*, respectively. This calculation can be repeated for various tracers, including stable isotope composition, ion concentrations, to calculate the relative contributions of various pollutants, ions and volumes of water. Combining these mass balance calculations allows estimation of the relative loads of pollution from both subcatchments, even without directly measuring the volumes of inflowing water, which is often challenging in the field.

A similar approach can be adopted for other subcatchments of the same order. The River Section *BBB* flows through an area with multiple industrial poultry farms. The potential influence of the farms on water quality in the river section *BBB* can be verified by comparing the results from $b3$ located downstream and $c1$ located upstream of the farms. The potential impact of the farms and the stable isotope signature of pollution can also be verified by sampling local drains ($b4$) or shallow groundwater bores ($x1$, $x2$ and $x3$). The potential impact of the mining site on water quality in the river section *DDD* can be verified by analysing the difference in water quality between sampling point $e1$ located upstream in relation to the mine and point $d3$. The influence of agrochemicals used for crop farming can be analysed by comparing the results from $b2$ and $d1$. Knowing the water quality change through each of the selected creek and river sections, the contributions to the catchment pollution budget can be further calculated using other triple mixing points ($d3 + d2 = d1$ for mixing between *DDD* and *KKK* and $b3 + b2 = b1$ for mixing *CCC* and *BBB).*

2.2 Other Input Data

The ranges of the stable isotope compositions typical of the various types of pollutants can be obtained from the literature; however, these values should be verified locally. If possible, the signatures of potential pollution sources should be analysed directly in the study area at the source of pollution (e.g., by obtaining fertilisers and discharged wastewater directly from animal farms or water draining from mining sites, etc.) (see also, Chap. 6).

The use of this simplifying sampling design template requires some understanding of the study area, particularly its hydrology and land use, and the acquisition of basic GIS information about the locations of potential sources of pollution. Any hydrochemical or hydrogeological information, if available, will help improve the interpretation of the results. The suggested multiple mixing models that cover different orders of catchments will allow the calculation of the relative contributions of the pollution from each listed source if the stable isotope signatures in the sources are significantly different. Understanding the local climate and obtaining rainfall records are also important factors. Fieldwork for hydrochemical studies is usually recommended during dry periods, at least a few weeks following substantial precipitation events. High-volume precipitation may dilute solute concentrations after the initial flushing down of pollutants accumulated and retained in water pools or soil. However, the major ion ratios and stable isotope signatures will not be directly impacted by dilution with rainwater if the concentrations of solutes are a few orders of magnitude higher than those in precipitation.

Reference

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Chapter 3
Principles of Mixing and Fractionation Models

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Abstract Upfront consideration of possible mixing scenarios is required to achieve an optimal sampling design and deliver a successful project. This will also help increase efficiency by reducing the number of samples, workload and cost without compromising the final outcomes. Chapter 2 summarised key information about a conceptual framework for devising a sampling design for tracing non-particulate agricultural pollutants in surface and groundwaters on a catchment scale. This chapter provides a short introduction to mixing models applicable to tracers for water pollution studies.

3.1 The Mixing Model Concept

The stable isotope composition of an element in a chemical compound dissolved in water (i.e., a solute) reflects its origin/source and, to some extent, the stable isotope fractionation processes that may have modified its initial signature during its transport and retention in solution. Failure to account for stable isotope fractionation processes may thus negatively affect the final conclusions of partitioning contributions from various sources of pollution.

A solute analysed in a water sample often originates from more than one source and therefore represents a mixture. This mixture is characterised by a mean stable isotope composition that is proportional to the relative contributions from all sources and the original stable isotope signatures of those sources. If the stable isotope signatures of individual sources are known and if they differ significantly from each other, then the stable isotope composition of the mixture can be used to estimate the relative contribution from each source. The calculation algorithm for this estimation is known as a stable isotope mass balance, and it can be applied to various elements in various solutes analysed in a single sample. However, an unambiguous solution of mass balance equations requires that the number of tracers used be not lower than one less

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than the number of sources under consideration (e.g., if three sources are contributing to a mixture, we need a minimum of two tracers; if four sources are considered, we need a minimum of three tracers, etc.). If fewer than the required number of tracers is available, these tracers can still be used for mass balance calculations, but an accurate calculation of the exact contributions will not be possible. Instead, possible ranges of contributions from each source can be obtained. The concept of mixing models is illustrated in the following three examples, which show different scenarios according to the different numbers of tracers available.

3.2 Calculation Examples

3.2.1 Example 1—Two-Source Mixing Using One Tracer

This is a simplified example of calculations for a two-source mixing model using only one stable isotope tracer (fractionation is not considered). The stable sulfur isotope composition, $\delta(^{34}\mathrm{S})$, has been analysed in a water sample collected from a river at a site below a large area of agricultural farmland where high sulphate concentrations were detected. Two sources of sulphate were considered to be major contributors to the elevated sulfate concentrations in the river ($\mathrm{SO_4}^{2-} = 800$ mg/L): (1) oxidation of pyrite ($\mathrm{FeS_2}$) that naturally occurred in the metamorphic rocks common in the study area and (2) potash fertiliser (potassium sulphate $\mathrm{K_2SO_4}$), applied in large quantities by farmers.

Input values
$\delta(^{34}\mathrm{S})_{\mathrm{Pyr}} = 1.5 \pm 0.5$ ‰; the mean signature of pyrite samples collected from several rock outcrops.
$\delta(^{34}\mathrm{S})_{\mathrm{Fer}} = 6.2 \pm 0.6$ ‰; the signature analysed in fertiliser samples collected from a few bags purchased by the farmers.

Mixture values
$\delta(^{34}\mathrm{S})_{\mathrm{Spl}} = 5.2$ ‰ the signature of sulfates in the river water sample.

Mass balance
The following set of simultaneous equations can be used for mass balance calculations of the relative contributions of fertiliser and pyrite to elevated sulfate concentrations in the river:

$$\delta\left(^{34}S\right)_{\mathrm{Spl}} = x_{\mathrm{Pyr}} \times \delta\left(^{34}S\right)_{\mathrm{Pyr}} + x_{\mathrm{Fer}} \times \delta\left(^{34}S\right)_{\mathrm{Fer}} \tag{3.1}$$

$$1 = x_{\mathrm{Pyr}} + x_{\mathrm{Fer}}, \tag{3.2}$$

where x_{Pyr} and x_{Fer} are the fractions of pollution ($\mathrm{SO_4}^{2-}$) from pyrite and fertiliser, respectively. Since only two sources are considered, the sum of x_{Pyr} and x_{Fer} is equal to 1 (by fraction) or 100 %; therefore, these equations can be easily solved, as follows:

$$x_{\mathrm{Fer}} = 1 - x_{\mathrm{Pyr}} \tag{3.3}$$

and, by substitution

$$\delta\left(^{34}S\right)_{\mathrm{Spl}} = x_{\mathrm{Pyr}} \times \delta\left(^{34}S\right)_{\mathrm{Pyr}} + \left(1 - x_{\mathrm{Pyr}}\right) \times \delta\left(^{34}S\right)_{\mathrm{Fer}} \tag{3.4}$$

and this is finally rearranged to

$$x_{\mathrm{Pyr}} = \frac{\delta(^{34}S)_{\mathrm{Spl}} - \delta(^{34})S_{\mathrm{Fer}}}{\delta(^{34}S)_{\mathrm{Pyr}} - \delta(^{34})S_{\mathrm{Fer}}}. \tag{3.5}$$

Using the input and mixture data given above in the example and Eq. 3.5, the following can be calculated:

$$x_{\mathrm{Pyr}} = \frac{5.2 - 6.2}{1.5 - 6.2} = 0.21 \tag{3.6}$$

$$x_{\mathrm{Fer}} = 1 - 0.21 = 0.79. \tag{3.7}$$

This means that the rock weathering and resulting pyrite oxidation contributes only to 21% of the total sulfate concentration of 800 mg/L observed in the river water, whereas the majority of the sulphate (79 %) originates from fertiliser. This can be translated into loads respective to concentrations of 168 mg/L originating from pyrite and 632 mg/L from fertiliser. If the outflow from the catchment is known, then the total load of sulfate originating from agricultural pollution discharged downstream can be estimated (e.g., mg/h or kg/day) by multiplying the outflow (e.g., L/h) by the concentration (e.g., mg/L). However, one drawback that must be kept in mind is that these numbers reflect the hydrochemical conditions at the time of sampling. As a result, they may change seasonally due to variability in hydrological conditions, the timing of fertiliser applications and whether crops are being planted or harvested. If more frequent (e.g., monthly) and long-term monitoring of the hydrochemical and stable sulfur isotope composition is conducted, and if the total annual load of fertiliser in the catchment is known, the annual agricultural pollution loads and the efficiency of fertiliser utilisation by plants can be estimated (see, e.g., Szynkiewicz et al. 2015a, b).

Note that for a two-source mixing model, the δ-value of the mixture must fall between the δ-values of the two considered sources (Fig. 3.1). If the δ-value of the mixture is outside the range covered by the δ-values of two sources, then the following scenarios are likely: (1) the sources have been incorrectly identified; (2) a third additional source is contributing to the mixture; or (3) the original stable isotope compositions of the sources have been significantly modified by stable isotope

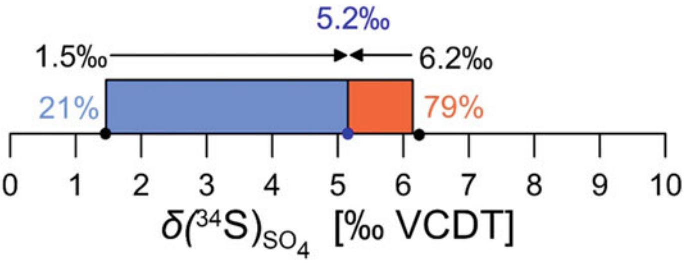


Fig. 3.1 Two-source mixing model concept (data as for Example 1). The $\delta(^{34}S)$ value of the mixture must lie between the $\delta(^{34}S)$ values of the sources. The size of the plotted bar is inversely proportional to the contribution from each source; therefore, a larger distance between the signature of the source and the signature of the mixture implies a lower contribution to the mixture

fractionation or equilibration processes, so the δ-values used are not a true reflection of the source signatures.

3.2.2 Example 2—Three-Source Mixing Using Two Tracers

More than two major sources often contribute simultaneously to water pollution. In these cases, more tracers need to be analysed in each sample to allow calculation of the exact contributions from individual sources (e.g., if three sources are considered, a minimum of two different isotope tracers need to be used). This simplified example considers a three-source mixing model using two stable isotope tracers (fractionation was not considered). The stable nitrogen and oxygen isotope compositions [$\delta(^{15}N)$ and $\delta(^{18}O)$] have been analysed in a water sample collected from a river outflowing from an agricultural area where high nitrate concentrations were detected. Three major NO_3^- sources were considered: precipitation, fertiliser and manure.

Input values

$\delta(^{15}N)_{Prec} = 5.2$ ‰ and $\delta(^{18}O)_{Prec} = 40.8$ ‰; the mean signatures of NO_3 analysed in precipitation.

$\delta(^{15}N)_{Fer} = -2.3$ ‰ and $\delta(^{18}O)_{Fer} = 5.0$ ‰; the mean signatures of NO_3 analysed in fertiliser.

$\delta(^{15}N)_{Man} = 25.0$ ‰ and $\delta(^{18}O)_{Man} = 5.4$ ‰; the mean signatures of NO_3 analysed in manure.

Mixture values

$\delta(^{15}N)_{Spl} = 16.9$ ‰ and $\delta(^{18}O)_{Spl} = 8.1$ ‰; the signatures of a water sample from the river.

Mass balance

Equations for mass balance calculations can be prepared in the same way as depicted in Example 1, assuming that the stable isotope compositions of both nitrogen and oxygen remain unchanged and fully identify the initial signatures of the sources.

$$\delta^{15}N_{\text{Spl}} = x_{\text{Prec}} \times \delta\left(^{15}\text{N}\right)_{\text{Prec}} + x_{\text{Fer}} \times \delta\left(^{15}\text{N}\right)_{\text{Fer}} + x_{\text{Man}} \times \delta\left(^{15}\text{N}\right)_{\text{Man}} \tag{3.8}$$

$$\delta\left(^{18}\text{O}\right)_{\text{Spl}} = x_{\text{Prec}} \times \delta\left(^{18}\text{O}\right)_{\text{Prec}} + x_{\text{Fer}} \times \delta\left(^{18}\text{O}\right)_{\text{Fer}} + x_{\text{Man}} \times \delta\left(^{18}\text{O}\right)_{\text{Man}} \tag{3.9}$$

$$1 = x_{\text{Prec}} + x_{\text{Fer}} + x_{\text{Man}} \tag{3.10}$$

where x_{Prec}, x_{Fer} and x_{Man} are the fractions of NO_3 originating from precipitation, fertiliser and manure, respectively. Since the contributions are assumed to arise from only these three sources, the sum of $x_{\text{Prec}} + x_{\text{Fer}} + x_{\text{Man}}$ is equal to 1 (by fraction) or 100 %. These simultaneous equations (Eqs. 3.8, 3.9 and 3.10) can be mathematically solved by substitutions.

The solution of this mass balance model, using the input and mixing data for Example 2, is as follows:

$$\begin{aligned} x_{\text{Prec}} &= 0.08 \\ x_{\text{Fer}} &= 0.24 \\ x_{\text{Man}} &= 0.68 \end{aligned}$$

In Example 2, the majority of the NO_3^- pollution detected in the river originates from manure, at 68 %, and fertilisers, at 24 %, whereas precipitation delivers only 8 %.

Note that for a three-source mixing model, the δ-value of the mixture must lie within the triangle (or polygon if more sources are considered) restricted by the δ-values of the three considered sources. The triangle shows the range of all possible δ-values which can originate from mixing of the considered sources proportionally to their contributions (Fig. 3.2).

3.2.3 Example 3—Three-Source Mixing Using One Tracer Only

Often, more than two major sources contribute to water pollution, but an inadequate number of tracers is available (e.g., if three sources are considered, but only one isotope tracer is available). In this scenario, the exact contribution from each source cannot be calculated. Instead, ranges of contributions may be calculated that can provide information about possible maximum and minimum contributions. Example 3 presents the same scenario with the same three sources discussed in Example 2, but only one tracer, $\delta(^{15}\text{N})$ has been used for the calculations.

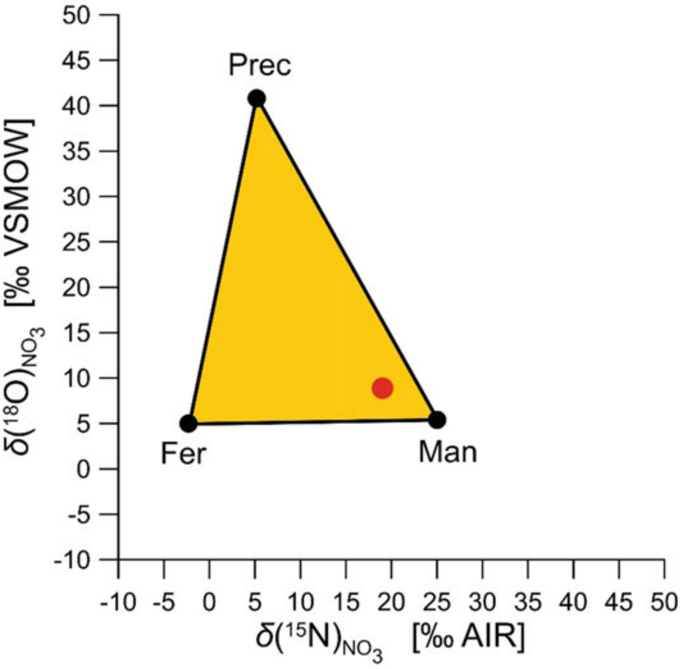


Fig. 3.2 Three-source mixing model concept (data as for Example 2). The signature of the mixture, in proportion to the contributed fractions, must fall between the signatures of the three sources; therefore, it will lie within the yellow mixing triangle. For simplification, fractionation was not considered in this example

Input values
$\delta(^{15}N)_{Prec} = 5.2$ ‰; a mean signature of NO_3 analysed in precipitation.
$\delta(^{15}N)_{Fer} = -2.3$ ‰; a mean signature of NO_3 analysed in fertiliser.
$\delta(^{15}N)_{Man} = 25.0$ ‰; a mean signature of NO_3 analysed in stored manure.

Mixture values
$\delta(^{15}N)_{Spl} = 16.9$ ‰; the signature of a water sample from the river.

Mass balance
The mass balance equations for this scenario cannot be mathematically solved because three unknowns cannot be calculated using only two equations. Instead, the ranges and the combination of values for solving these equations can be obtained using, for example, IsoSource software (Phillips and Gregg 2003) (See input data in Appendix 2).

$$\delta\left(^{15}N\right)_{Spl} = x_{Prec} \times \delta\left(^{15}N\right)_{Prec} + x_{Fer} \times \delta\left(^{15}N\right)_{Fer} + x_{Man} \times \delta\left(^{15}N\right)_{Man} \tag{3.11}$$

$$1 = x_{Prec} + x_{Fer} + x_{Man} \tag{3.12}$$

where x_{Prec}, x_{Fer} and x_{Man} are fractions of NO_3 originating from precipitation, fertiliser and manure, respectively. Since the contributions from three sources are considered, the sum of $x_{\mathrm{Prec}} + x_{\mathrm{Fer}} + x_{\mathrm{Man}}$ is equal to 1 (by fraction) or 100 %.

The solution of this mass balance model, using the input and mixing data for Example 3, is as follows:

$$\begin{aligned} X_{\mathrm{Prec}} &= 0 \text{ to } 0.41 \quad (\text{mean } 0.20) \\ x_{\mathrm{Fer}} &= 0 \text{ to } 0.30 \quad (\text{mean } 0.15) \\ x_{\mathrm{Man}} &= 0.59 \text{ to } 0.70 \quad (\text{mean } 0.65). \end{aligned}$$

These calculations do not provide exact values; however, the obtained information can still be very useful as it confirms that the major source of nitrate in the polluted river is manure and that its contribution is not lower than 59 % and not higher than 70 % with mean 65 %. The remaining contribution, between 15 and 20 %, originates from fertiliser and precipitation, which can vary from 0 to 41 % and 0 to 30 %, respectively.

The mixing models all assume good mixing of water and pollution, but this is not always the case. Inhomogeneity may introduce additional uncertainty into mass balance calculations. In addition, none of the examples presented considered stable isotope fractionations. A fractionation may occur during chemical reactions, physical phase changes, or biological processes, particularly if these processes are not completed and not all substrates are exhausted. The effects of stable isotope fractionations can be corrected, but this will require more advanced modelling and obtaining specific fractionation factors for each stable isotope, chemical compound and physicochemical condition at the time of the reaction (e.g., Lewicki et al. 2022). All results, calculations and interpretations reflect the conditions at the time of sampling, and these can vary over seasons and can be particularly impacted by changes in the hydrological conditions (Dogramaci and Skrzypek 2015; Szynkiewicz et al. 2015a; Dogramaci et al. 2017).

3.3 Overview of Available Software for Mass Balance and Fractionation Models

Simple mixing models that use the number of tracers lower by one than the number of sources ($t = \mathrm{s}-1$) can be solved algebraically and do not require advanced software. The solution can be derived from simultaneous equations, as presented in examples 1 and 2 above, and the final equation is then solved manually or implemented in a calculation spreadsheet. These options will provide an exact mathematical solution, and the uncertainty of the final calculation will be associated with the laboratory analyses and accurate determination of the signatures of sources and mixtures, but not with the calculation formula that provides the exact solution. The algebraic solution is not applicable in cases where fewer tracers are available ($t \leq s-2$). That situation usually requires the calculation of a range of possible combinations of values solving equations, or alternatively, a probability calculation can be attempted. A few models have been published in recent years using different calculation methods, although most of these models have been developed for assessing dietary contributions in food web studies (e.g., Phillips et al. 2014). Although they were intended for ecological studies, they can easily be adopted in pollution studies. Here, the most popular models are presented: IsoSource, MixSIAR and FRAME.

3.3.1 IsoSource—Algebraic Solution for Complex Models

The IsoSource mixing model was developed to allow the calculation of the proportional contributions from a large number of sources using an algebraic algorithm. The software (v 1.3.3) works on Microsoft Windows systems (including Windows 10) and accepts input values for up to five tracers/isotopes and for a maximum of ten sources. All possible scenarios are calculated at user-defined increments and tolerances. The results are returned as a table with fractions of the relative contributions that solve the mixing model equations. Minimum, maximum and mean values are calculated and allow the determination of the possible range of contributions from each source. The software allows the calculation of fractions for one mixture at a time and does not assess uncertainty. The results are recorded as separate report files and need to be combined manually by the user. The system is easy to use, and all inputs are entered manually into a provided window. IsoSource does not have the option of uploading input files.

Website: https://www.epa.gov/eco-research/stable-isotope-mixing-models-estimating-source-proportions
Primary reference: Phillips and Gregg (2003)
Alternatives: LP_Tracer (Bugalho et al. 2008); Moore–Penrose pseudoinverse (Hall-Aspland et al. 2005); SOURCE/STEP (Lubetkin and Simenstad 2004).

3.3.2 MixSIAR—Bayesian

MixSIAR is an R package that uses Bayesian statistical methods for (1) determining the proportional contribution of different sources to a mixture and (2) assessing dispersion in the isotope space (Stock et al. 2018). This package, like its earlier version, SIAR, and like IsoSource, was developed for stable isotope studies of food webs. The first functionality (1) can be easily adopted for any mixing model, whereas the second functionality (2), although it is used to assess the distribution of stable isotope results, is specific for ecological studies and not directly applicable to pollution studies. Bayesian statistical distributions are used for both the characterisation of source contributions and uncertainties. The package is a ready-to-use product and could be used in R-Studio, but elementary knowledge of R coding is required. The model accepts input files, and batches of data can be processed.

Website: http://brianstock.github.io/MixSIAR/
Primary reference: Stock et al. (2018)
Alternatives: MixSIR (Moore and Semmens 2008; Ward et al. 2010); SIAR (Parnell et al. 2010); IsotopeR (Hopkins and Ferguson 2012) and FRUITS (Fernandes et al. 2014), isoWater (Bowen et al. 2018).

3.3.3 FRAME—Bayesian, Markov Chain Monte Carlo

FRAME (isotope FRactionation And Mixing Evaluation) is a newly developed software that works on the Windows platform. The FRAME mathematical algorithm uses the Markov chain Monte Carlo model to estimate the contribution of individual sources and processes, as well as the probability distributions of the calculated results. It has a user-friendly graphical interface that can simultaneously determine mixing proportions and the progression of the fractionating process. The fractionation can be defined by the user for specific requirements of the particular isotope system (e.g., an open or closed system). The model can integrate up to three isotopic signatures for each compound used to identify sources contributing to the mixture. This model has been specifically developed for the assessment of mixing and emissions in agriculture pollution studies; therefore, it can be used for any mixing model for pollution studies. The software is easy to use, and datasets can be uploaded using the provided spreadsheet templates.

Website: https://malewick.github.io/frame/
Primary reference: Lewicki et al. (2022).
Alternatives: isoWater (Bowen et al. 2018) (https://cran.r-project.org/web/packages/isoWater/index.html)

3.3.4 Other Models and Calculation Spreadsheets

A few other calculation spreadsheets and models can be applicable to agro-contaminant studies (e.g., Hydrocalculator, EasyIsoCalculator). Many studies require estimations of different processes, including evaporative losses in different parts of a catchment, retention times, or evaporation over inflow ratio in lakes or dam reservoirs. Evaporative loss also informs about the evapo-concentration of solutes and can confirm evaporation as a process driving high concentrations of solutes. These calculations are rather too complex to solve manually and can be easily preformed using Hydrocalculator (Skrzypek et al. 2015).

Tracer studies that use compounds with artificially elevated concentrations of heavier isotopes are used to track pollution dispersal or chemical uptake by biological organisms. In these studies, stable isotope results are reported using different expressions (e.g., fraction, ratio or delta). The EasyIsoCalculator spreadsheet allows fast and effortless recalculation between the main expressions (Skrzypek and Dunn 2020a, b).

3.3.5 Hydrocalculator—Estimating Evaporative Losses

The Hydrocalculator software (v 1.3) works on the Microsoft Windows system and allows the calculation of evaporative losses from a water body using the stable hydrogen and oxygen isotope composition of the water. The mathematical algorithm is based on a modified version of the Craig–Gordon model. The user can choose between a steady-state and non-steady-state model and select one of three options for estimating the stable isotope composition of atmospheric moisture. The software has an easy-to-use graphical interface. The data can be entered manually in calculator mode or uploaded as a template file for batch conversion. Outputs are recorded in a spreadsheet file (CSV, comma-separated values).

Website: http://hydrocalculator.gskrzypek.com
Primary reference: Skrzypek et al. (2015).
Alternatives: FRAME (Lewicki et al. 2022), isoWater (Bowen et al. 2018).

3.3.6 EasyIsoCalculator—Recalculating Between Fractions, Delta Values and ppm

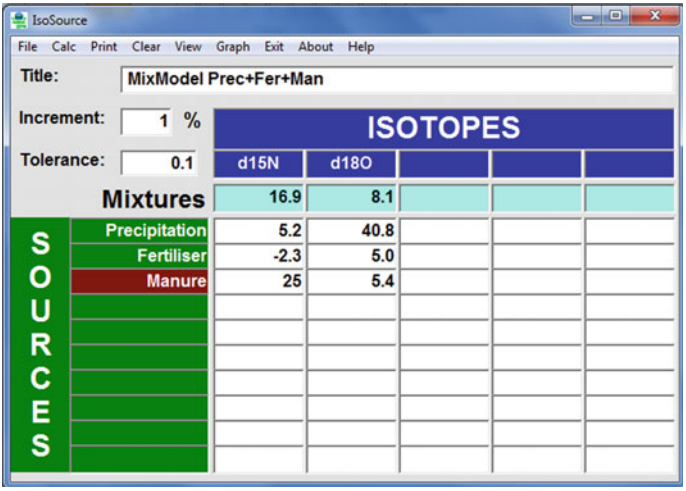
EasyIsoCalculator is a calculation spreadsheet that allows recalculation of the main expressions of isotope compositions (isotope ratio, fraction and delta) for five light elements: hydrogen, carbon, nitrogen, oxygen and sulphur. The spreadsheet allows the selection of one of many absolute ratios defining zero points on isotope delta scales. The spreadsheet has an easy-to-use interface with dropdown menus, and its open code allows the addition of new values. Up to fifty results can be calculated at once using the copy-and-paste option.

Website: http://easyisocalculator.gskrzypek.com/
Primary reference: Skrzypek and Dunn (2020a, b)
Alternatives: n/a.

Appendix 1

How to use IsoSource software (data as for mixing model, Example 2, presented in Chap. 3).

1. Download and install the software from https://www.epa.gov/eco-research/stable-isotope-mixing-models-estimating-source-proportions (Philips and Gregg 2003).
2. Start the software and enter the $\delta(^{15}N)$ and $\delta(^{18}O)$ data in the table, inputting values for your water sample into the blue cells, and values for pollution sources into the white cells (increment 1 % and tolerance 0.1).



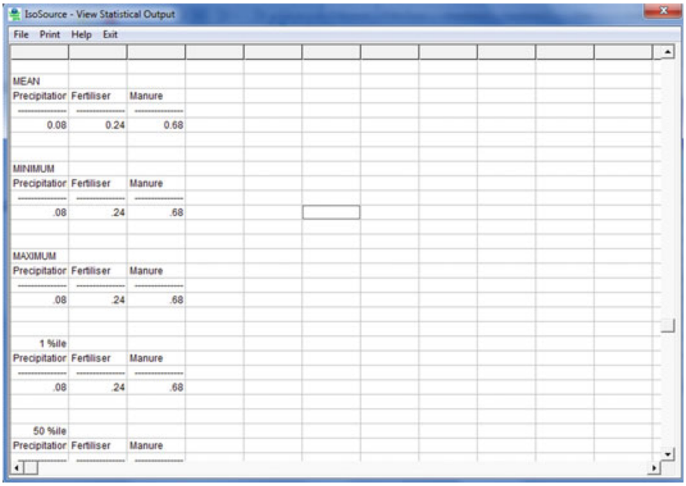
Title: MixModel Prec+Fer+Man

Increment: 1 %

Tolerance: 0.1

	d15N	d18O			
Mixtures	16.9	8.1			
Precipitation	5.2	40.8			
Fertiliser	-2.3	5.0			
Manure	25	5.4			

3. Click ‘Calc’ on the upper toolbar and save your result file in your folder using the name of a sample as the name of your file.
4. Now you can open your results. Click View (a new window will pop up) and then File.
5. Open the file containing your results of calculations with extension *.tot (e.g., samplename.tot)
6. The results are at the bottom of the file. For this particular example, the mean, maximum and minimum contributions are equal.

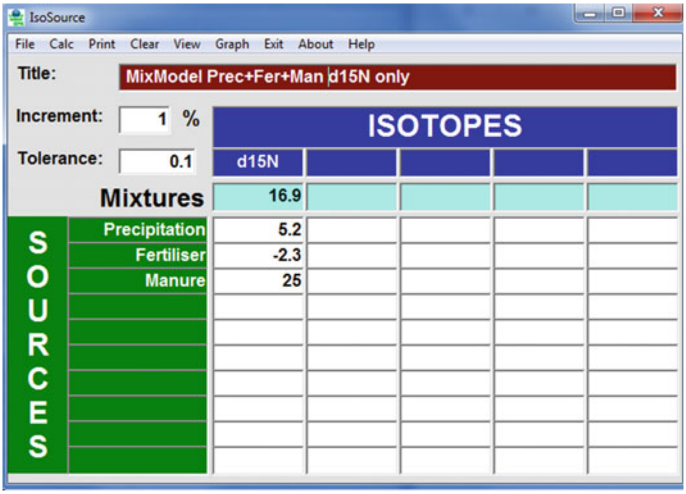


```
MEAN
Precipitatior Fertiliser  Manure
------------- ----------- -----------
         0.08        0.24        0.68

MINIMUM
Precipitatior Fertiliser  Manure
------------- ----------- -----------
          .08         .24         .68

MAXIMUM
Precipitatior Fertiliser  Manure
------------- ----------- -----------
          .08         .24         .68

       1 %ile
Precipitatior Fertiliser  Manure
------------- ----------- -----------
          .08         .24         .68

      50 %ile
Precipitatior Fertiliser  Manure
------------- ----------- -----------
```

7. Transfer or type your results into an appropriate table in an Excel spreadsheet.
8. Calculate for each sample separately, one sample at the time.

Appendix 2

1. Input Values for IsoSource Software from Example 3 in Chap. 3



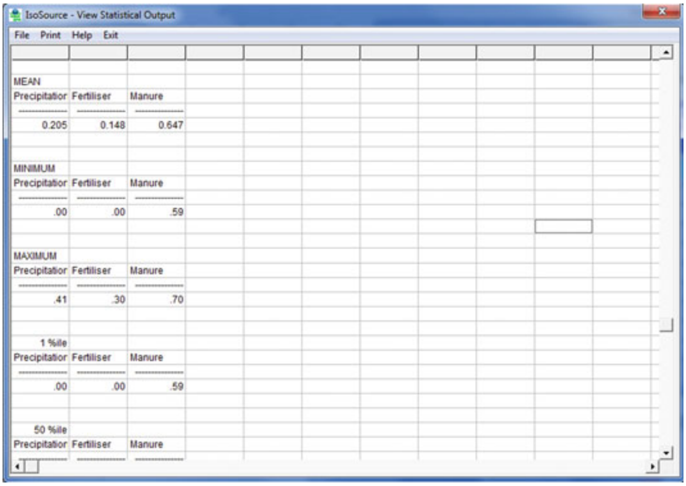
Title: MixModel Prec+Fer+Man d15N only

Increment: 1 %

Tolerance: 0.1

	d15N
Mixtures	16.9
Precipitation	5.2
Fertiliser	-2.3
Manure	25

2. The results are at the bottom of the *.tot file and show the possible ranges for each source, which solves Eqs. 3.11 and 3.12. Calculate for each sample separately, one sample at the time.



```
MEAN
Precipitatior Fertiliser   Manure
-----------   -----------  -----------
      0.205        0.148        0.647


MINIMUM
Precipitatior Fertiliser   Manure
-----------   -----------  -----------
        .00          .00          .59


MAXIMUM
Precipitatior Fertiliser   Manure
-----------   -----------  -----------
        .41          .30          .70


      1 %ile
Precipitatior Fertiliser   Manure
-----------   -----------  -----------
        .00          .00          .59


     50 %ile
Precipitatior Fertiliser   Manure
-----------   -----------  -----------
```

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Grzegorz Skrzypek Grzegorz has 24 years of experience in the field of stable isotope geochemistry, hydrochemistry and ecology since his PhD (1999) from The University of Wrocław (UWr). Grzegorz is currently working as Associate Professor and Coordinator of MSc Biology Ecology specializations at The University of Western Australia (UWA). Grzegorz has published many papers on applications of stable isotope techniques in hydrological, hydrochemical, pollution, ecological and paleoclimate studies. He uses stable H, C, N, O, S, and Sr isotopes to trace water budget, nutrient fluxes in natural environments, and dispersal of pollution in environments impacted by mining and agriculture. He held a prestigious Australian Research Council Future Fellowship at UWA (2012-2015) and Foundation for Polish Science Scholarship at UWr (1999-2000). Currently, he is serving as the deputy director of the West Australian Biogeochemistry Centre at UWA (wabc.uwa.edu.au). Grzegorz has been a chief investigator on a number of research projects in Europe and Australia and an expert on IAEA Technical Cooperation Projects and IAEA Technical Meetings.

Chapter 4
Compound-Specific Isotope Analyses to Investigate Pesticide Transformation in Soil and Water

T. Gilevska and G. Imfeld

Abstract This chapter provides an overview of approaches employed in tracking pesticide degradation within agricultural catchments, focusing on the existing challenges and burgeonic prospects afforded by pesticide compound-specific isotope analyses (CSIA). The discussion centers on the development of CSIA for low concentrations of pesticides in environmental matrices. Additionally, the chapter explores the viability of implementing pesticide CSIA in field applications for tasks such as for source apportionment, discerning transformation reactions, and quantifying the extent of degradation on a catchment scale.

4.1 Overview of Pesticide Dissipation Processes in Soil and Water

The transport and distribution of pesticides in the environment are determined by numerous factors, including the physicochemical properties of the active compounds, the mechanisms of formulation, the rate of application, as well as local conditions such as topography, the soil and vegetation characteristics, and the hydro-climatic conditions. Pesticide behaviour in soil and water is determined by chemical, physical, and biological processes, such as sorption–desorption, volatilization, degradation, uptake by plants, and leaching, all of which contribute to pesticide dissipation, which includes degradative and non-degradative processes. The relative significance and cumulative effect of these processes vary according to both pesticide and matrix properties. Transformation of pesticides involves both biotic processes (e.g., microbial degradation) and abiotic processes (e.g., photochemical reactions). The contribution, rate, and extent of the transformation processes for a given pesticide are determined by its chemical structure and environmental conditions. While evaluating the specific contributions of pesticide transformation pathways is essential for risk assessment and decision-making on the catchment scale, robust approaches

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to characterize and constrain these processes pesticide transformation are mostly missing.

For instance, physicochemical properties of the soil (pH and SOM content, redox gradients in soils), biological properties (activity and distribution of microorganisms), and environmental conditions controlling soil temperature and moisture generally can affect both biotic or abiotic transformations. However, within soils biotic transformation of pesticides typically dominates over abiotic transformation, e.g., photochemical transformations which are typically restricted to only the top submillimetre layers of soil. In contrast, in shallow surface waters, phototransformation (with indirect photolysis being more relevant to pesticide degradation) can significantly contribute to pesticide degradation. For some pesticides, e.g., organophosphate pesticides, hydrolysis can also be a relevant abiotic reaction in the aqueous phase. However, for most pesticides, hydrolysis requires specific conditions such as high or low pH or low-redox environments. Given the multitude of processes influencing pesticide dissipation in the environment, accurate tracking of degradation as the primary process for removing parent compounds necessitates an approach grounded in multiple lines of evidence. This approach should encompass the integration of various complementary methods, which are briefly described below.

4.2 Evaluating Pesticide Transformation in Soil and Water: Current Approaches and Limitations

4.2.1 Pesticide Concentration Analysis

The rapid development of analytical instrumentation has enabled the detection and monitoring of multiple pesticide compounds in soil and water at low environmental concentrations (Alder et al. 2006; He and Aga 2019). Prior to analysis, different methods are commonly used to extract and concentrate pesticides from environmental samples, including solid-phase extraction (SPE) for water and quick, easy, cheap, effective, rugged, and safe procedure (QuEChERS) for soil (Anastassiades et al. 2003; Bonansea et al. 2013). Analyses by gas chromatography-mass spectrometry (GC–MS) or liquid chromatography-tandem mass spectrometry (LC–MS/MS) are the two most common methods for monitoring pesticide transformation in soil and water.

Pesticide concentration analysis is typically used in routine pesticide monitoring (e.g., by environmental agencies) and for examining in situ pesticide degradation. For instance, the concentration-based approach of monitoring 76 pesticide residues in 317 soil samples across the European Union revealed that over 80% of the soil samples contained pesticide residues (Silva et al. 2019). The fungicide boscalid was the most common pesticide molecule detected. Indeed, boscalid is very persistent in the soil matrix, with a dissipation half-life (DT_{50}) ranging from 130 to 337 days (Karlsson et al. 2016). However, while monitoring approaches based on concentration analysis alone enable quantification of pesticide residues in the environment, they remain limited in distinguishing between physical and degradative processes, both of which can contribute to overall pesticide dissipation. Consequently, a comprehensive framework to examine pesticide transformation and dilution in the environment, and especially process-based information, is mostly lacking. Although a substantial volume of data on pesticide degradation has been gathered through regulatory testing, the capacity to anticipate and quantify the extent of degradation and the specific pathways involved in various field conditions still poses a considerable challenge (Fenner et al. 2013).

Incorporating an examination of not only the parent pesticide molecules but also their transformation products (TPs) offers a valuable approach to relate concentration monitoring and the degradation processes taking place in the field, thus enabling a more comprehensive identification and quantification of these in situ processes. Additionally, TPs may alter the ecotoxicological effects of pesticides on ecosystems. Through targeted and non-targeted methods, i.e., used to characterize chemical structures of unknown compounds, this complementary analysis is thus fundamental to evaluate the environmental fate and effects of pesticides. TPs analysis is easier when TPs are known, and more challenging when non-target analyses are used. Nevertheless, recent advances in the interpretation of the non-target analysis allow for the identification of previously unidentified TPs. For instance, suspect screening of 242 pesticide TPs combined with national monitoring data has recently identified previously undetected TPs, even in the absence of the parent compound (Menger et al. 2021). However, this approach introduces additional complexity, as TPs are liable to not only be produced but degraded as well in a natural environment. Hence, even when TP-based analyses are applied, quantifying degradation based on concentration alone is subject to large uncertainty. Concentration monitoring of both parent compounds and their TPs is frequently integrated with numerical modeling approaches to simulate the degradation of parent compounds and the subsequent production and breakdown of resulting TPs within a specific study site.

4.2.2 Modelling Approaches

Computational models can be used to predict variables related to pesticide persistence and mobility in soil and water. As a result, they provide a means to describe and predict the reactive transport of pesticides with various degrees of uncertainty based on the input parameters (Goumenou et al. 2021). Numerous studies have attempted to model the behaviour and distribution of pesticides in the environment, ranging from the micro-environment to the global scale. Different modelling approaches have been evaluated in several review papers (Mottes et al. 2014; Wang et al. 2019). Typically, in most models, degradation is one of the most sensitive model parameters determining the fate of pesticides in soil and losses to surface water and groundwater (Dubus et al. 2003). Accurately capturing the dynamics of pesticide and TPs degradation is therefore particularly critical in the context of risk assessment using fate models since this can introduce considerable uncertainty into model predictions (Lindahl et al. 2008). In addition, scaling up pesticide dissipation processes requires conceptual simplification of localized processes with a minimal loss of information (Imfeld et al. 2021). For instance, understanding the relationship between degradation rates and measured field parameters (e.g., microbial activity, temperature and soil moisture) allows for the application of pesticide fate models at larger scales (Ghafoor et al. 2011). Reasonable constraints on these field parameters can be made by iterating between model development, sampling, and measurement schemes. In recent years, the Soil and Water Assessment Tool (SWAT) (Arnold et al. 1993) has been proposed as a comprehensive modelling technique to relate processes affecting pesticides in sediment–water interfaces to catchments and river basins (Imfeld et al. 2021).

4.2.3 Biomolecular Analysis

The structural and functional characteristics of microbial communities are also potential indicators for monitoring and evaluating the degradation of a given pollutant in the environment. Following environmental DNA (eDNA) extraction, mainly quantitative polymerase chain reaction (qPCR) and functional gene arrays can be used to examine the response of microbial communities to exposure to pesticides and the potential of microbial population to adapt and degrade specific pesticides (Fenner et al. 2013). However, both techniques rely on knowledge of the biologically driven pesticide degradation pathway and gene sequence coding, which are not typically available (Imfeld and Vuilleumier 2012). In addition, detection and quantification of pesticide-degrading genes from environmental DNA generally feature the genetic potential for pesticide degradation but not its expression. In some cases, RNA transcripts may help follow up target active microorganisms involved in the degradation process (Monard et al. 2013). However, RNAs remain difficult to retrieve and conserve from complex environmental samples such as soil. In future, next-generation approaches may build on approaches involving specific degradation genes and their expression to also include patterns of microbial diversity using metagenomics and meta-transcriptomics (Jeffries et al. 2018; Rodríguez et al. 2020; Malla et al. 2022). This could be applied to both probe the effect of pesticides on soil or water microorganisms, as well as to evaluate the pesticide degradation functions.

Beyond these approaches, compound-specific stable isotope analysis (CSIA) already offers a complementary approach with untapped potential to evaluate the sources and transformations of pesticides in the environment.

4.2.4 Compound-Specific Stable Isotope Analysis (CSIA)

4.2.4.1 Principles

Degradation processes (biotic or abiotic) typically lead to changes in the ratio of stable isotopes (e.g., $^{13}C/^{12}C$) in organic molecules. This change is related to the kinetic isotope effect (KIE) and depends on the preferential rate of bond breakage for weaker bonds (those with light isotopes, e.g., ^{12}C for carbon) compared to bonds bearing a heavy isotope (^{13}C for carbon). This may lead to an enrichment in the remaining contaminant in ^{13}C relative to the initial isotope composition (Hunkeler et al. 1999; Meckenstock et al. 1999; Sherwood Lollar et al. 1999). The isotope effects produced during physical processes, such as volatilization, dissolution, dispersion, and sorption are typically smaller, usually insignificant (relative to analytical uncertainty) compared to the large KIE that may be produced by bond cleavage (Hunkeler et al. 2008; Kuntze et al. 2020 and reference therein). Therefore, change of $^{13}C/^{12}C$ isotope ratios during pesticide degradation can provide direct evidence of its degradation. The CSIA approach has been extensively applied over the past two decades to measure and quantify the degradation of legacy pollutants in industrial-contaminated sites. Industrial sites, unlike agricultural fields, are typically characterized by very high concentrations of the pollutants due to the presence of non-aqueous phase liquids (Hunkeler et al. 2008).

4.2.4.2 Application of CSIA

To quantify the magnitude of the isotope fractionation during the given degradation process, isotope fractionation factors (ε) need to be derived in the laboratory. To do so, change in the isotope signatures, e.g., $\delta(^{13}C)$, can be related to the extent of degradation with the Rayleigh equation (Eq. 4.1):

$$\ln\left(\frac{\delta(^{13}\mathrm{C})_t + 1}{\delta(^{13}\mathrm{C})_0 + 1}\right) = \varepsilon \times \ln(f), \tag{4.1}$$

where $\delta(^{13}C)_0$ and $\delta(^{13}C)_t$ represent the isotope signatures at time 0 and t of the degradation, respectively, whereas f is the fraction of the remaining pesticides at time t (Fig. 4.1a).

Accurate determination of ε values is key to quantifying degradation processes in situ. From different forms of the Rayleigh distillation equation standard linear regression without forcing through zero is preferred (Scott et al. 2004) (Fig. 4.1b).

The extent of biodegradation (%) of an organic compound can be calculated in situ using the determined ε according to Eq. 4.2, without concentration data, eliminating the influence of physical processes.

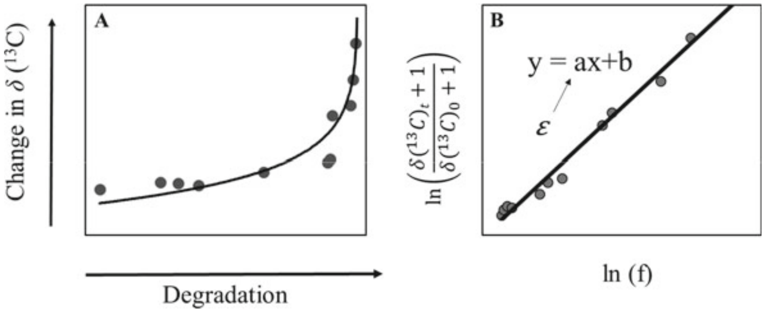

Fig. 4.1 **a**—Change of stable carbon-isotope compositions during degradation; **b**—Rayleigh equation for the same dataset

$$B = \left(1 - \left(\frac{\delta(^{13}\mathrm{C})_t + 1}{\delta(^{13}\mathrm{C})_0 + 1}\right)^{\frac{1}{\varepsilon_{\mathrm{bulk,C}}}}\right) \times 100 \qquad (4.2)$$

For GC- and LC-isotope ratio mass spectrometry (IRMS) analysis, organic compounds are converted into a simple gas for which the isotope ratio is determined (e.g., CO_2 for CSIA of carbon, N_2 for CSIA of nitrogen). Due to the total conversion of the molecule to a measuring gas, position-specific changes in the isotope composition of a target compound cannot be ascertained. Moreover, the isotope fraction is diluted with any additional atom that is not directly involved in the reaction. In the case of large molecules due to the number of carbon atoms per molecule, a large extent of degradation is required to measure significant stable carbon-isotope fractionation ($\Delta(^{13}\mathrm{C}) > 2$ ‰) to identify degradation in the field (Hunkeler et al. 2008). The isotope fractionation criterion for positive identification of in situ degradation of $\Delta(^{13}\mathrm{C}) > 2$ ‰ is typically established based on total analytical uncertainty (± 0.5 ‰), incorporating accuracy and reproducibility (Sherwood Lollar et al. 2007), which may vary among compounds and elements analysed.

Even fractionation experiments under conditions controlled in the laboratory can be influenced by several phenomena, including additional rate-limiting steps, transport across the cell membrane (Renpenning et al. 2015; Ehrl et al. 2019), low substrate bioavailability (Sherwood Lollar et al. 2010), or substrate-enzyme binding (Mancini et al. 2006). These rate-limiting steps can "mask" the ε normally associated with the particular bond-cleavage involved. In some cases, this can lead to an underestimation of isotope fractionation, making it difficult to determine the appropriate ε to use for quantifying the biodegradation of contaminants.

This highlights the relevance of multi-element CSIA (ME-CSIA) to circumvent the limitation of only one element CSIA approach. In theory, all elements in the molecule are affected in a similar way by the masking processes mentioned above, eliminating the bias observed for ε.

4.2.4.3 Multi-element CSIA (ME-CSIA)

ME-CSIA can provide additional support for interpreting in situ results, specifically when complex pollution scenarios with multiple pollution sources or different degradation processes occur. Many pesticides contain nitrogen atoms (e.g., carbamates, acetanilides, thiabendazoles, triazoles), and therefore nitrogen CSIA in addition to carbon CSIA may help tracing pesticides degradation. In such a case, transformation mechanisms are identifiable from dual-isotope plots [e.g., $\delta(^{13}\mathrm{C})$ versus, e.g., $\delta(^{15}\mathrm{N})$], reflecting underlying carbon- and nitrogen-isotope effects. For example, C-N dual-CSIA allows the identification of degradation mechanisms of six chloroacetanilide and acylalanine pesticides during abiotic hydrolysis (Masbou et al. 2018a). In ME-CSIA lambda (Λ) is the slope of the dual-isotope plot, and it reflects the changes in the stable isotope composition of one element versus the second element, which can be more specific to a reaction, and thus inform about transformation processes in the laboratory or in the field. The York method, which incorporates uncertainty in both variables (Ojeda et al. 2019), better adapts to the wide set of data conditions observed for dual-isotope data, and the natural logarithmic form is used for large isotope fractionation (Höhener and Imfeld 2021) (Fig. 4.2.).

Laboratory derived ε and Λ values for various pesticides and different degradation pathways can be found in recent review papers (Elsner and Imfeld 2016; Kuntze et al. 2020; Cui et al. 2021; Won et al. 2021). Nevertheless, CSIA datasets for pesticides during biotic and abiotic degradation processes are still currently limited, mainly due to analytical challenges (see below).

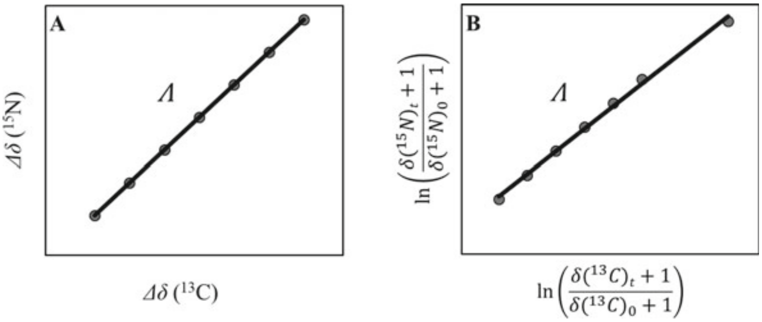

Fig. 4.2 Dual-isotope plot of **a**—raw Δ values according to (Ojeda et al. 2019) and **b**—ln-transformed δ values according to (Höhener and Imfeld 2021) for the same dataset

4.3 Compound-Specific Isotope Analysis (CSIA) of Pesticides in Soil and Water

4.3.1 Potential of Pesticide CSIA

Current research on pesticides focuses on toxicity, degradability, degradation pathways, and the formation of transformation products. However, novel management strategies relying on the natural and engineered degradation of pesticides and following the precautionary principle are warranted. Synthetic pesticides generally undergo degradation tests prior to use. The recurrent detection of herbicides in groundwater and soil emphasizes the difficulties in extrapolating laboratory tests to environmental conditions. Beyond the outcome of regulatory testing, current approaches are limited in their ability to (i) predict over relevant long time scales the degradation of micropollutants in the environment, (ii) evaluate, beyond environmental monitoring, the prevailing dissipation zones and periods (“hot/cold spots and moments”) in integrative studies on the catchment scale (Elsner and Imfeld 2016). Pesticides CSIA can provide additional assistance in this regard.

Although the analytical challenges currently limit field studies, including pesticide CSIA, applications in the environment are now within reach and can be foreseen in different scenarios of increasing complexity:

- *Point source scenario.* The fate of organochlorine pesticides, such as hexachlorocyclohexane (HCH), has been evaluated in several contaminated field sites with point source pollution using CSIA (Bashir et al. 2015; Chartrand et al. 2015; Liu et al. 2017, 2021; Wu et al. 2018, 2019a; Qian et al. 2019). In analogy to steady-state assessments of legacy contaminants, continuous pesticide release from a single point source related, for example, to pesticide spills during production, can be studied.
- *Event-based scenario.* Usually, pesticide pollution in agricultural fields does not conform to single point source scenarios. Instead, it often manifests as pesticide pulses, which can be traced in event-based studies to elucidate the dynamics of “hotspots” and “hot moments” (e.g., Riml et al. 2013). The isotope data of pesticides in surface water may enhance the interpretation of transformation pathways in heterogeneous reactive compartments. The first field studies have recently been reported (Schreglmann et al. 2013; Alvarez-Zaldivar et al. 2018).
- *Large-scale studies.* Comprehensive studies on the catchment scale may also integrate multiple pesticide sources and events over multiple seasons (Moschet et al. 2013). Pesticide CSIA may thus also serve comprehensive studies on a larger spatial scale with multiple sources and events over a season but may miss where, when, and how degradation occurs. For example, the stable carbon-isotope signature was used to evidence the migration and degradation of DDTs in a large-scale study of arable soils across China (Niu et al. 2016). In this respect, although multi-element CSIA may also be desirable to further improve characterization,

its feasibility is challenged by the sampling window, where quantification limits can be achieved.

Additionally, the combination of passive sampling and pesticides CSIA opens novel opportunities. Passive sampling techniques have been widely used to integrate mean concentrations of organic contaminants over extended sampling periods (< 50 days) and to pre-concentrate micropollutants, allowing their detection in trace amounts in surface waters (10–50 pg/L). A passive sampling approach combined with CSIA has been previously applied for vapour intrusion studies of legacy contaminants, such as benzene (Goli et al. 2017), and has recently been tested for pesticide in wetlands in agricultural fields (Gilevska et al. 2022). $\delta(^{13}C)$ and $\delta(^{15}N)$ of pesticides in Polar Organic Chemical Integrative Sampler (POCIS) deployed in surface water would reflect their degradation in both water and hydrologically connected soil. Therefore, when compared to the initial isotope signature of the pesticide formulation applied, isotope signatures from POCIS can indicate degradation in both water and soil in the entire agricultural catchment.

Complementarily, pesticide chirality can provide yet another tracer to understand the fate of chiral pesticides in the environment. One-third of marketed pesticides are chiral, and most of them are used as racemates, despite the fact that the desired activity usually depends on one enantiomer, while the other(s) has adverse environmental effects (Maia et al. 2017). As many degradation processes are enantioselective, monitoring changes in the enantiomeric fraction (EF) can be used to follow degradation in situ. Furthermore, combining CSIA and enantioselective analysis techniques for the enantioselective stable isotope analysis (ESIA) may enhance the evaluation of sources and transformation processes of individual pesticide enantiomers in the environment (Milosevic et al. 2013; Elsner and Imfeld 2016; Jin and Rolle 2016; Masbou et al. 2018b, 2023). For example, combined evidence from concentrations, enantiomer ratios, and isotope composition confirmed the degradation of the herbicide dichlorprop and its metabolite in the hotspot at the contaminated site in Denmark (Milosevic et al. 2013).

4.3.2 Challenges of Pesticide CSIA

The occurrence of very low (sub-µg/L) concentrations of pesticides and their polarity are two major analytical challenges to be addressed to expand CSIA approaches to pesticides. First, the enrichment of sufficient analyte (typically a few ng of C or N on column per injection are needed) requires the extraction of large amounts of water, soil, or vegetal material. The detection limits for nitrogen CSIA of pesticides are much higher than for carbon, as typically there are fewer atoms of N than C in the pesticide molecule, making it difficult to apply ME-CSIA in situ (Elsner and Imfeld 2016). Any scaling up of the extraction method should be monitored for potential isotope effects. As shown by Melsbach et al. (2021), increasing the volume of water during SPE above 10 L may alter the $\delta(^{13}C)$ of atrazine.

For accurate and precise CSIA, complete chromatographic separation of all compounds is required. Therefore, any chromatographic interferences can strongly limit the applicability of the CSIA. As the majority of pesticides are non-volatile or semi-volatile on-column liquid injection is typically used as a sample introduction technique when pesticides are analysed with GC-IRMS. This technique, in contrast to static or dynamic headspace sampling used for volatile organic compounds, can introduce many matrix interferences from compounds with similar physicochemical properties from soil or water co-enriched during extraction procedures. For example, during large-volume SPE, non-volatile matrix components from environmental samples are thus enriched together with the target compounds, which is defined here as the matrix effect. Extract clean-up procedures are thus often necessary prior to the application of CSIA to environmental samples (see Sect. 4.4.2.3).

The second challenge lies in the polarity of the more polar pesticides and their transformation products. This generally requires the use of a derivatization step prior to GC separation or the use of LC-IRMS, which is currently limited to $\delta(^{13}C)$ analyses. LC-IRMS is further constricted due to high detection limits (at least one order of magnitude higher than GC-IRMS). LC-IRMS has also limited application due to the incompatibility of the method with organic solvents, such as methanol, typically used for chromatographic separation (Gilevska et al. 2014; Perini and Bontempo 2021). Therefore, the most common derivatization step is chosen to circumvent the polarity of the parent compound or daughter products. The choice of the derivatization method strongly depends on the chemical structure of the pesticide. Methylation of hydroxyl and amino groups is typically achieved with trimethylsilyldiazomethane (TMSD) and trimethylsulfonium hydroxide (TMSH) (Reinnicke et al. 2010; Mogusu et al. 2015; Melsbach et al. 2019; Torrentó et al. 2019). Any additional step, such as derivatization, requires strict screening for any isotope fractionation during the sample preparation. Additionally, the stable carbon-isotope composition should be corrected if additional carbon atoms are introduced during derivatization.

4.4 Soil and Water Sample Preparation and Measurements with GC-IRMS for Stable Carbon- and Nitrogen-Isotope Composition of Pesticides

4.4.1 Sampling Strategy

The following sampling strategy is suggested to quantify pesticide degradation extent under field conditions in small agricultural catchments (i.e., 10–100 ha). Both sampling and interpretation should be adapted for larger scales to include multiple sources and events over an agricultural season.

First, sufficient knowledge regarding hydrological and hydro-climatic conditions and functioning is mandatory for the application of pesticide CSIA at the catchment scale (Table 4.1). Hydro-climatic data should provide sufficient resolution to evaluate

(i) mean daily rainfall, (ii) mean rainfall intensity, (iii) total rainfall, (iv) mean daily reference evapotranspiration, (v) mean daily temperature, (vi) mean daily discharge normalized by the total catchment area, (vii) time of concentration, and (viii) proportion of days in a month when rainfall occurred (% Wet Days). Subsurface travel time should be defined precisely, possibly with preliminary hydrological studies using the stable hydrogen and oxygen isotope composition of water [$\delta(^{2}H)$ and $\delta(^{18}O)$], refer to Sect. 6.3.1.

The soil sampling frequency should be adapted to the pesticide application and the expected degradation kinetics. Transects should be selected to account for soil type, heterogeneity, and the variability of moisture conditions, drainage characteristics and to maximize the number of plots where the pesticide is applied. In addition, a digital elevation model (DEM) may be used to determine local slopes and to estimate the topographical wetness index (TWI) [-]. TWI mainly quantifies the impact of topography on soil moisture. Soil crust development should be characterized across the catchment after a precipitation event as a function of mm of cumulative rainfall. This should allow us to evaluate the reduction in the soil infiltration capacity due to crusting, and to interpret the temporal evolution of TWI along with rainfall-runoff data.

Table 4.1 Typical sampling scheme for water and soil samples for implementing pesticide CSIA on the catchment scale

	Prior to sampling	Measurements and analyses	Sampling
Water	*Hydrological and climatic data* • Daily precipitation and temperature data • Contribution of surface, subsurface, and base-flows • Concentration time of the catchment	• Discharge measurements • Multiparameter probes in situ • Hydrochemical analysis	Flow proportional sampling with refrigerated automatic sampler with glass vials Applied commercial pesticide formulations
Soil	*Pedological and surface soil data* • Pedological characterization • Digital elevation model • Agricultural plots (owners, limits, etc.) • Typical agricultural scheme	• Farmers survey for the period of pesticide application • Plant growth and coverage • Soil moisture characteristics • Soil physicochemical characterization • Erosion budget	Topsoil (1 cm) and/or discrete subsurface soil (in different layers from 0 to 20 cm or deeper) across several transects Applied commercial pesticide formulations

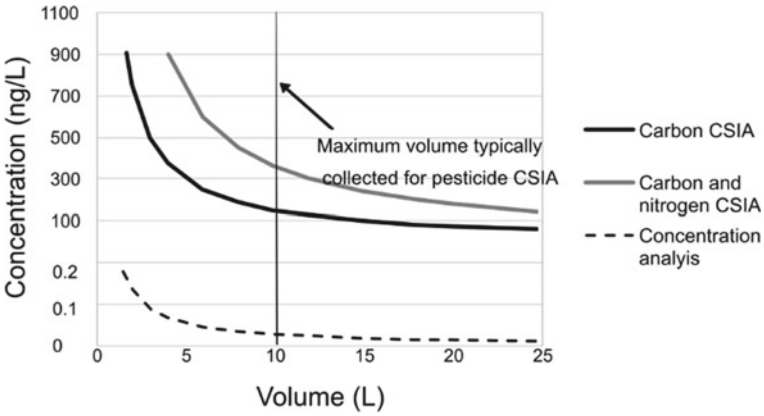

Fig. 4.3 Relationship between the necessary *S*-Metolachlor concentrations in water and collected water volume for reliable carbon or nitrogen CSIA and concentration analysis (Alvarez-Zaldivar et al. 2018; Torrentó et al. 2019). The solid black line represents feasibility of carbon CSIA, solid grey line represents feasibility of carbon and nitrogen CSIA, dashed line represents feasibility of concentration analysis. Note a different scale for the concentration analysis. Note that this relationship is site-specific

During sampling, the water discharge at the catchment outlet should be continuously monitored to evaluate hydrological functioning and to establish water and pesticide mass balances. After sampling, the sub-samples of water may be pooled into composite samples according to hydrograph characteristics and pesticide concentrations, yielding one or more samples weekly with the chosen water volume. This volume should be selected to ensure that the concentrations of each pesticide are sufficient for CSIA analysis. An example of the sample size and the potential for pesticide and isotope analysis is illustrated in Fig. 4.3.

4.4.2 Water and Soil Sample Processing and Conservation

Water samples should be collected using a refrigerated autosampler in the field, stored in the dark at 4 °C during collection (to avoid photolysis and limit further biodegradation), and placed on ice during transportation to the laboratory for immediate filtering (on 0.7 or 0.45 μm glass fibre). Water samples should be kept at 4 °C from the collection up to the extraction and should be preferably further filtered and analysed (using SPE, see Sect. 4.4.3) within 24 h. Preliminary tests on the effect of water sample sampling, transport, and storage should be done prior to the study. Water samples may also be frozen for longer-term conservation, but preliminary tests of the effect of freezing on both extraction yield and stable isotope fraction are required for each targeted compound.

After collection, soil samples should be kept in an ice box during transport to the laboratory and kept frozen at −20 °C until analysis. Soils should be homogenized, quartered before sieving (e.g., according to NF X 31,100 standard), and sieved at 2 mm. Water content, pH, organic content, CEC, and other parameters can be measured to characterize soil samples and help in data interpretation.

4.4.3 Extraction Methods for CSIA from Water and Soil

To expand the use of pesticide CSIA to agricultural catchments, it is crucial to use an appropriate extraction method. Extraction methods for pesticide residues from environment matrices for ME-CSIA should: (i) provide sufficient analyte mass for reliable isotope analysis, (ii) cause no isotope fractionation [$\Delta(^{H}E)$], (iii) be applicable to a wide range of pesticides and matrices, (iv) limit matrix co-enrichment to avoid co-elution during chromatographic separation.

4.4.3.1 Extraction Methods from Water

To date, SPE has been the most common method used to extract water samples for pesticide CSIA although liquid–liquid extraction has been used for small amounts of water (from 0.5 mL) in laboratory experiments (Chevallier et al. 2018; Knossow et al. 2020). The extraction methods should be tested within the expected concentration range and target environmental matrix to determine the feasibility of pesticide CSIA from water samples. SPE has been tested previously in combination with CSIA of atrazine, acetochlor, *S*-Metolachlor, metalaxyl, butachlor, alachlor, terbutryn, chlordizon, bentazone, dichlorvos, dimethoate, omethoate and several of their metabolites (Schreglmann et al. 2013; Elsayed et al. 2014; Wu et al. 2014; Schürner et al. 2016; Masbou et al. 2018a; Torrentó et al. 2019; Drouin 2021; Droz 2021; Pérez-Rodríguez et al. 2021). To ensure maximum recovery, the type and quantity of sorbents and extraction eluents should be adjusted to the physical properties of the analytes and matrix. As the physicochemical properties of parent compounds and their TPs may differ significantly, different sorbents or sorbent combinations may be used to pre-concentrate the parent compounds and the TPs (Torrentó et al. 2019).

The majority of pesticide CSIAs studied did not show isotope fractionation using SPE (Schreglmann et al. 2013; Elsayed et al. 2014; Wu et al. 2014; Masbou et al. 2018a; Torrentó et al. 2019; Droz 2021; Pérez-Rodríguez et al. 2021). A sample size of up to 10 L is more frequently used for SPE and pesticide CSIA, as increasing the volume > 10 L could change the carbon isotope ratios $\delta(^{13}C)$ of pesticides (Melsbach et al. 2021). However, this change may not be correlated with the SPE procedure but rather with the matrix effect on the measurement, decreasing oxidation capacity, and increasing background levels and instrument maintenance issues. Therefore, prior to the pesticide measurements, clean-up strategies (discussed below) are typically applied to minimize the matrix effect. Nevertheless, current SPE methods allowed carbon and nitrogen pesticide CSIA in the ng/L to μg/L of pesticide concentration range (Schreglmann et al. 2013; Alvarez-Zaldivar et al. 2018; Torrentó et al. 2019). This underscores the feasibility of carbon and nitrogen CSIA from water samples in agricultural settings throughout the agricultural season.

4.4.3.2 Extraction Methods from Soil and River Sediment

When choosing an extraction method and solvent, one should take into account the physicochemical properties of pesticides, including their hydrophobicity and acid dissociation constant, as well as soil properties like pH, organic matter content, and moisture levels. For optimal results, extraction tests must be carried out with the studied soil or sediment to ensure high recovery and non-significant isotope fractionation and evaluate the matrix effect due to the co-extraction of soil organic matter. Modifications to existing methods and protocols, such as increasing the sample volume from 5 to 20 g or sequential use of extraction solvents, should also be evaluated.

Pesticide extraction methods from soil and sediment for reliable pesticide CSIA have been already used in both laboratory and field studies (Alvarez-Zaldivar et al. 2018; Masbou et al. 2018b; Wu et al. 2019b; Pérez-Rodríguez et al. 2021). Ivdra et al. (2014) proposed a modified ultrasonic-assisted extraction (MUSE) without carbon-isotope fractionation associated with extraction [$\Delta(^{13}C) \leq 0.4$) for hexachlorocyclohexanes (HCHs). A modified MUSE method (Ivdra et al. 2014) was also tested with ethylacetate (Alvarez-Zaldivar et al. 2018; Masbou et al. 2018b) or dichloromethane:pentane (Droz et al. 2021; Pérez-Rodríguez et al. 2021) as the extraction solvent. Another study used accelerated solvent extraction (ASE) for the extraction of HCHs from soil and plants, which enabled stable C, H, and Cl isotope analysis (Wu et al. 2019b; Liu et al. 2020, 2021). The application of QuEChERS for the extraction of metolachlor from two agricultural soils led to an insignificant (< 1 ‰) isotope fractionation for carbon CSIA (Torrentó et al. 2021).

Current methods allow for carbon and nitrogen CSIA in soil samples in the range of ng/g to μg/g range for carbon and several μg/g for nitrogen CSIA (Alvarez-Zaldivar et al. 2018; Masbou et al. 2018b; Droz 2021). This currently restricts the application of carbon and nitrogen CSIA of pesticides to source areas and laboratory studies, respectively. In order to reduce concentration ranges for ME-CSIA, there is a need for simple and fractionation-free purification techniques that can be applied to a wide spectrum of environmental soils and pesticides.

4.4.3.3 Clean-Up Procedures

A number of clean-up procedures can be applied to address the issue of co-enrichment to maximize the analytical performance of pesticide CSIA extraction from environmental matrices without altering the isotope ratio of the target compounds. These include: (i) the addition of a sorbent, such as primary secondary amine or graphitized carbon black, to remove pigments, such as chlorophyll (Anastassiades et al. 2003; Wilkowska and Biziuk 2011), (ii) chromatography HPLC separation or column chromatography (Schreglmann et al. 2013; Mogusu 2016), and (iii) the use of molecularly imprinted polymers (MIP) (Bakkour et al. 2018). MIP is likely the most effective clean-up method for CSIA. However, MIP is not commercially available for all classes of compounds and therefore must be specifically synthesized prior to clean-up. Furthermore, samples should only be processed in glass when using organic solvents, especially dichloromethane, to reduce matrix interferences from the extraction procedure.

Carbon and nitrogen CSIA in complex matrices can also benefit from two-dimensional gas chromatography (GC × GC)-IRMS. This approach has been applied to polychlorinated biphenyls, chloronaphthalenes, and chlorofluorocarbons (Horii et al. 2005; Horst et al. 2015). In GC × GC-IRMS, the system needs to be equipped with a column-switching device, such as moving capillary stream switching (MCSS), or with a 6-port valve. By column-switching parts of the effluent from the first column are cut and transferred to a second column, where separation of compounds of interest can be enhanced. In addition, the use of (GC × GC)-IRMS can improve sensitivity by eightfold (Horst et al. 2015) by enhancing chromatographic separation. Therefore, this approach has potential for pesticide CSIA application.

Whenever possible, one or several clean-up strategies, depending on the complexity of the matrix, should be applied during pesticide CSIA. Along with improving the background of pesticide CSIA, it will reduce the need for increased maintenance of the GC-IRMS instrument, including oven and column replacement as well as blockage of capillaries. It is also possible to cut the first 10–20 cm of the GC column if some small chromatographic interferences are observed in blanks preventing retention of the entire column (be aware of the change in retention time and the measurement windows). Complementarily with clean-up procedures, it may be possible to extend the temperature programme to improve the level of background and chromatographic separation when the background compromises the GC-IRMS measurements. However, this will increase the measurement times.

It is worth mentioning that pesticide CSIA must be preceded by quantitative evaluation of the pesticides in the extracts to optimize injections of the target analytes. Prior CSIA, rigorous quality assurance practices and referencing strategies must be established to ensure that isotope measurements are accurate and reproducible.

4.4.4 *Optimal GC-IRMS Conditions for Pesticide CSIA*

4.4.4.1 Referencing Strategies

An important component of quality assurance is the use of analytical standards of pesticides calibrated on international isotope scales. The referencing strategy of the "identical treatment principle" (Werner and Brand 2001) should be implemented: (i) to measure external standards before and after the sample to correct for an offset, and (ii) to use a reference material that is identical to the target substance. To obtain in-house isotopic standards, a mass spectrometer equipped with an elemental analyzer and isotope ratio mass spectrometer (e.g., Flash EA IsoLinkTM CN IRMS, Thermo Fisher Scientific, Bremen, Germany) or offline conversion followed by dual-inlet-IRMS are typically used. The $\delta(^{13}C)$ and $\delta(^{15}N)$ values should be normalized to VPDB and Air scales, respectively, using a minimum of two international reference materials with the range of isotope δ values, that would encompass all the measured samples, are chosen as an anchor point for the regression line, e.g., IAEA600 [$\delta(^{13}C) = -27.77$ ‰] and USGS41 [$\delta(^{13}C) = +37.63$ ‰] (Coplen et al. 2006). Then, the third standard, which has the value between the chosen anchors is treated as an unknown sample for quality control (QC), and is used to evaluate combined analytical uncertainty (Coplen 1988). As a part of inter-laboratory comparison, it is recommended to measure the same standards at other lab facilities using another EA-IRMS.

Unless otherwise specified, the uncertainties for pesticide CSIA are reported as standard deviation (1σ) calculated from replicate measurements. The combined analytical uncertainty of $\delta(^{13}C)$ and $\delta(^{15}N)$ values should not differ (≤ 0.5 ‰) from values obtained by EA-IRMS.

The standard injection frequency depends on the pesticides, the element measured, the matrix, and the number of pesticides analysed. For relatively simple environmental matrices (e.g., groundwater), it is recommended to measure an in-house pesticide mix standard with known isotope composition at least every six samples to control the retention time of the target compounds and assess the instrument performance (e.g., conversion efficiency). The standard mixtures are injected every three samples, however, for simultaneous $\delta(^{15}N)$ analysis of several pesticides for complex environmental matrix (e.g., soil). Peak amplitudes of injected standards should closely match those of analysed samples (Sherwood Lollar et al. 2007). To conserve the combustion capacity of the oven, the measurement window (effluent mode) should be kept to a minimum: opened before the targeted peak elution and closed shortly thereafter.

As part of CSIA method validation, the linearity range should be determined for each pesticide. The linearity range specifies the range of measurements with sufficient precision and accuracy, indicating, within an acceptable range [e.g., ± 0.5 ‰ for $\delta(^{13}C)$], that the stable isotope composition is independent of the amount of compound injected (Jochmann et al. 2006). The method detection limit (MDL) is the point with the lowest (or highest) concentration within a ± 0.5 ‰ linear interval of the

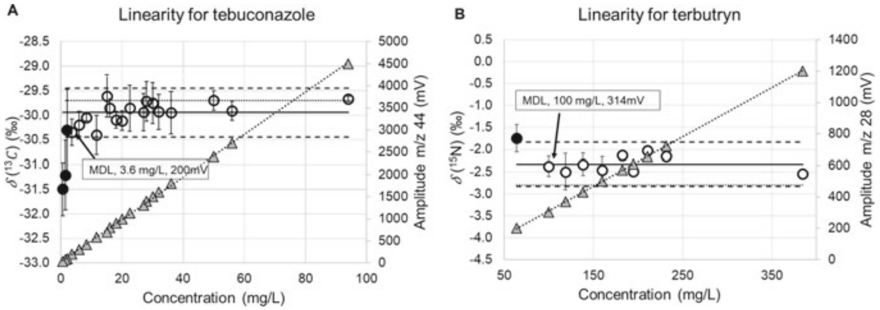

Fig. 4.4 Concentration and A—$\delta(^{13}C)$ measurements and amplitude of the mass 44 for tebuconazole B—$\delta(^{15}N)$ measurements and amplitude of the mass 28 for terbutryn. Circles represent stable carbon- and nitrogen-isotope compositions. Triangles indicate the amplitude of mass 44 and 28 peaks. The solid line represents the calculated mean $\delta(^{13}C)$ value (– 29.9 ± 0.2 ‰, $n = 51$) and $\delta(^{15}N)$ value (– 2.3 ± 0.2 ‰, $n = 30$); dashed lines indicate the ± 0.5 ‰ interval. Measurements were performed in triplicate; the standard deviation of each point is indicated by error bars. The dotted line represents the $\delta(^{13}C)$ value of tebuconazole (– 29.7 ± 0.1 ‰, $n = 3$) and $\delta(^{15}N)$ value of terbutryn (– 2.8 ± 0.1 ‰, $n = 3$) measured by EA-IRMS. Values outside the linear range-filled circles are excluded from the mean $\delta(^{13}C)$ and $\delta(^{15}N)$ value calculation due to either being outside ± 0.5 ‰ interval from the mean value or due to low reproducibility of triplicate measurement (> 0.5 ‰). MDL—method detection limit. The major principles illustrated in this figure are described in (Jochmann et al. 2006)

mean value for the standard measured with GC-IRMS and with good reproducibility in triplicate measurements (< 0.5 ‰). In Fig. 4.4a shows the linearity for $\delta(^{13}C)$ for pesticide tebuconazole, with three data points (filled circles) outside ± 0.5 ‰ linear interval from the mean value or with low reproducibility of triplicate measurements (> 0.5 ‰). Such measurements are not taken into consideration and are considered to be outside the linear range. Figure 4.4b shows the linearity for $\delta(^{15}N)$ for terbutryn, with MDL corresponding to a significantly higher concentration of the compound in the sample.

4.4.4.2 Conversion to gas

Organic compounds are converted to a simple measuring gas CO_2 for carbon CSIA and N_2 for nitrogen CSIA. For both carbon and nitrogen CSIA, the conversion can be achieved in a combustion reactor comprised of, for example, NiO tube, CuO, NiO, and Pt wires and operated at 1000 °C (P/N 1255321, GC IsoLink II IRMS System, Thermo Fisher Scientific). Within the combustion oven, two reactions occur for nitrogen (some setups use two separate units for oxidation and reduction) (Eq. 4.3).

$$R - NH_2 \rightarrow^{\text{Oxidation}} N_2 \overset{\text{Reduction}}{\leftarrow} N_xO_y \qquad (4.3)$$

During nitrogen CSIA, the produced CO_2 is trapped downstream using a liquid nitrogen trap to reduce measurement interference. Pesticides containing heteroatoms, such as N and Cl, and more complex chemical structures pose challenges to the combustion process, resulting in incomplete conversion and lower sensitivity. CSIA of nitrogen is particularly challenging, since the heavier isotope ^{15}N has a lower natural abundance (0.04%) than carbon heavier isotope ^{13}C (1.1%), and fewer heavy nitrogen atoms are present in organic substances. Moreover, two N atoms are required to form N_2, and N_2 has a lower ionization efficiency. To obtain a similar precision to that of $\delta(^{13}C)$ analysis, theoretically a 50 times higher sample amount is required for $\delta(^{15}N)$ analysis, leading to high substance loads that can affect conversion efficiency (Reinnicke et al. 2012). To enhance combustion during pesticide CSIA, different modifications were proposed, combining changes in reactor design, reactor temperature, and oxidation pattern. The variation of these parameters can be optimized if a large offset or low reproducibility is observed during carbon or nitrogen pesticide CSIA. A self-made Ni/NiO reactor operated at 1150 °C (Meyer et al. 2008), a NiO tube/CuO–NiO reactor operated at 940 °C (Reinnicke et al. 2012), a Ni/Ni/Pt reactor operated at 1000 °C (Spahr et al. 2013) have been used to enhance conversion of different pesticides during carbon and nitrogen CSIA.

During the oxidation procedure, oxygen is pumped into the reactor in backflash mode, replenishing the oxygen required for conversion. For nitrogen, excess oxygen might lead to incomplete reduction of N_xO_y species into N_2. The oxidation time should be adjusted to achieve a balance between sufficient oxygen for the quantitate conversion but not too much, so that reduction is not hindered.

4.5 Towards Field Studies Using Pesticide CSIA and Interpretation of Stable Isotope Signatures

Integrative strategies relying on isotope data have been conducted for more than three decades in the case of nitrate and have significantly contributed to the development of water management policies (Nestler et al. 2011; Lutz et al. 2013; Moschet et al. 2013). A similar strategy has been developed for legacy pollutants (Hunkeler et al. 2008) and may be now developed for pesticides in agricultural catchments, as recently described (Alvarez-Zaldivar et al. 2018).

Prior to the sampling campaign, which would include pesticide CSIA information about applied pesticides, application period, published ε and Λ values for pesticides used, the isotope signature of the pesticide formulation should be gathered.

4.5.1 Insights from Pesticide CSIA

4.5.1.1 Occurrence of in Situ Degradation and Pathway Identification

When $\Delta(^{13}C)$ or $\Delta(^{15}N) > 2$ ‰ (assuming combined analytical uncertainty of ± 0.5 ‰) of pesticide molecules is observed across space, time, or source (i.e., compared to the isotope signatures of pesticides in applied commercial formulations), pesticide degradation is likely occurring in situ. Further, the degradation pathways may be identified based on the determined ε and Λ values. For example, in the biotic hydrolysis of atrazine by *Arthrobacter aurescens* TC1, an unusual trend towards more negative $\delta(^{15}N)$ values is observed. Protonation of nitrogen atoms during the reaction makes ^{14}N react more rapidly while ^{15}N accumulates in the remaining atrazine (inverse isotope effect) (Meyer et al. 2009). In contrast, oxidative dealkylation by *Rhodococcus* sp. strain NI86/21 would result in more positive $\delta(^{15}N)$ values. As mentioned in Sect. 4.3, the use of dual element isotope plots is preferred, especially when multiple sources are present.

4.5.1.2 Quantitative Assessment of in Situ Degradation

When the degradation pathway is identified in the field, then Eq. 4.2 can be used to calculate the extent of biodegradation. For pesticides, these calculations typically require a large extent of degradation to fulfil $\Delta(^{13}C) > 2$ ‰ requirement. For example, for *S*-Metolachlor (using $\varepsilon_C = -1.5 \pm 0.5$ ‰ for biodegradation in soil (Alvarez-Zaldivar et al. 2018; Droz et al. 2021), the extent of degradation of the applied pesticide should be higher than 74% to apply carbon CSIA to identify and quantify degradation in situ. However, owing to the multitude of processes influencing pesticide residues in agricultural fields, such as contamination pulses during runoff events, these estimates of the extent of biodegradation are likely to be conservative. Therefore, a subsurface-surface reactive transport model incorporating CSIA isotope source ratios, ε values for different elements can help examine sources and dissipation of pulses of diffuse contaminants (Van Breukelen 2007; Lutz et al. 2017).

4.5.2 Pesticide Database for Source Identification and Apportionment

The initial stable isotope composition of organic chemicals depends on the conditions and the pathways used to synthesize the compound and thus depends on the manufacturers and the time frame of production. This variation may serve to identify chemical sources or trace the time of contaminant release in the environment. The range of the initial isotopic ratios of these compounds from various manufacturers is thus needed to trace pesticide origin and dissipation.

Table 4.2 Range of $\delta(^{13}C)$ of *S*-Metolachlor for a standard (PESTANAL®) and four different pesticide formulation measured by GC-IRMS

	PESTANAL®	Dual Gold SAFENEUR®	CAMIX 440®	MERCANTOR GOLD®	S-Metolastar®
$\delta(^{13}C) \pm$ uncertainty (‰)	− 31.0 ± 0.5	− 31.9 ± 0.2	− 31.7 ± 0.2	− 32.6 ± 0.5	− 32.2 ± 0.2

Therefore, prior to applying pesticide CSIA in the field, the isotopic composition of commonly or formerly used pesticides produced by different manufacturers from different regions should be determined for different elements. Relevant pesticide commercial formulations (1–5 mL in clean glass vials stored at 4 °C or at − 20 °C until analysis) can be collected directly at the farms or following official requests to regional and national pesticide providers and manufacturers. The active compounds of the different pesticide formulations can be isolated by either liquid–liquid extraction or measured directly by GC-IRMS. For example, Table 4.2. shows the narrow range of $\delta(^{13}C)$ of *S*-Metolachlor for four different pesticide formulations (from − 31.7 ± 0.2 ‰ to − 32.6 ± 0.5 ‰) and slightly different composition of an analytical standard (− 31.0 ± 0.5 ‰). Based on that range, if the $\delta(^{13}C)$ of *S*-Metolachlor measure in situ is around − 29.0 ‰, it may indicate degradation.

The ISOTOPEST database (https://ites.unistra.fr/isotopest) was established to address the need for a comprehensive repository of stable isotope signatures of pesticides in commercial formulations. Its primary purpose is to enable seamless inter-laboratory comparisons and foster the utilization of stable isotope data in forthcoming research endeavors concerning pesticide transformation and source tracking in the environment.

4.5.3 Identifying Isotope Fractionation During Degradation in Microcosm Experiments

Degradation laboratory experiments under controlled conditions must clearly demonstrate the relationship between concentrations decrease and changes of stable isotope composition, and identify the prevailing degradation pathway in specific environmental compartments (Hunkeler et al. 2008). Several studies have determined the range of ε and Λ values for various pesticides (Masbou et al. 2018a; Cui et al. 2021; Drouin et al. 2021; Torrentó et al. 2021; Won et al. 2021). However, with the increasing number of commercial formulations, it is likely that specific information concerning isotope fractionation during degradation may not be available for most pesticides. Additionally, field-specific information is preferred for the most accurate identification and quantification of the degradation process. Reference hydrolysis, photolytic, or biodegradation experiments under laboratory-controlled conditions can be carried out to derive field-specific ε and Λ values for pesticides from water, sediment, or soil samples. It is possible that photodegradation may be a relevant process in the first soil mm or water surface (Cui et al. 2021; Drouin et al. 2021). Reference degradation experiments may ideally be carried out with an isolated strain degrading metabolically the targeted pesticide, together with the identification of TPs and targeted microbial assessment (i.e., specific functional genes and/or taxa associated with degradation) to characterize degradation in the field using the multi-line-of-evidence approach.

4.5.4 Outlook

The requirements, bottlenecks, and possible solutions at various scales and levels of complexity to apply pesticide CSIA to field-based studies are summarized in Table 4.3. In most cases, field applications have focused on point source scenarios of persistent pesticides, which typically have higher concentrations and therefore require smaller sample sizes and present fewer measurement challenges (Hunkeler et al. 2008). Degradation is typically assessed by comparing the source isotope signature over time and space. Such applications can be combined with ME-CSIA, ESIA, and microbial analysis to identify the prevailing degradation processes (Bashir et al. 2015; Chartrand et al. 2015; Liu et al. 2017, 2021; Wu et al. 2018, 2019a; Qian et al. 2019). Additionally, the implementation of high-resolution CSIA approaches can provide further constraints on characterizing the dynamics of active degradation zones and compartments (e.g., sediment–water interfaces or soil surface) (Gilevska et al. 2021).

For pesticide CSIA application to event-based scenarios, a detailed sampling strategy must be developed based on possible sources, hydrology, and land use (see Sect. 4.4.1). Additionally, isotope data can be interpreted within a framework that includes physicochemical and hydrological tracer data to evaluate and quantify pollution sources and the processes that the pesticide has undergone during its retention and transport in a catchment (Elsner and Imfeld 2016). In order to monitor the degradation of pesticides effectively, it is imperative to conduct sampling prior to

Table 4.3 Requirements, bottlenecks, and possible solution of pesticide CSIA application for different scenarios

Scenario	Requirements	Bottlenecks	Possible solutions	References
Point source	• ε and Λ values for different degradation processes • Hydro-climatic conditions • Isotope signature at the source area	• Multiplicity of degradation processes	• Application of ME—CSIA • Combination of pesticide CSIA with ESIA, concentration analysis of parent compound and TPs, microbial analysis	(Bashir et al. 2015; Chartrand et al. 2015; Liu et al. 2017, 2021; Wu et al. 2018, 2019a; Qian et al. 2019)

(continued)

Table 4.3 (continued)

Scenario	Requirements	Bottlenecks	Possible solutions	References
Event base	• ε and Λ values for different degradation processes • $\delta(^{13}C)$ and $\delta(^{15}N)$ of the applied pesticides • Hydro-climatic conditions • Survey of pesticide application periods	• Low concentration of pesticides • Short contamination event can be missed • Lack of published ε and Λ values • Multiplicity of degradation processes	• Sampling campaign should begin before the expected application period to measure background concentration and isotope signatures of remaining pesticides, during the application, and after to follow the degradation of pesticides • Large sample volumes should be collected in case of low environmental concentration (e.g., up to 10 L for water samples) • Automatic proportional sampling or application of POCIS would capture short contamination events • Field-specific ε and Λ experiments • Combination of pesticide CSIA with ESIA, concentration analysis of parent compound and TPs, microbial analysis, and modelling approaches	(Schreglmann et al. 2013; Lutz et al. 2017; Alvarez-Zaldivar et al. 2018)
Large scale	• ε and Λ values for different degradation processes • Database of different source signatures • Hydro-climatic conditions of catchment(s) • Survey of pesticide application periods	• Complexity of multiple pesticide sources, application events, and variability of site-specific characteristics • Lack of published ε and Λ values • Low concentration of pesticides	• Pesticide isotope data should be interpreted together with physicochemical and hydrological tracer data to evaluate the pollution sources and transport in a catchment • Combination of pesticide CSIA with ESIA, concentration analysis of parent compound and TPs, microbial analysis, and modelling approaches • Several field-specific ε and Λ experiments	(Niu et al. 2016)

the pesticide application period. This initial sampling serves the crucial purpose of quantifying background concentrations and isotopic signatures of residual pesticides. Establishing this accurate baseline is essential as it provides a reference point for the assessment of both short-term and long-term impacts following pesticide application. Given that diffuse pesticide pollution typically leads to low environmental concentrations, the collection of substantial and representative volumes of water and soil becomes imperative for the reliable implementation of pesticide CSIA. This need for comprehensive sampling is particularly significant in cases where nitrogen isotopic analysis is involved. In addition, a monitoring strategy incorporating this complementary analysis requires narrower sampling time frames to ensure sufficient sampling resolution to capture short-duration high concentration episodes, such as large runoff events. In future, integrative sampling with passive samplers (e.g., POCIS) can be implemented to ensure that such events will be sampled at sufficiently high resolution. It can be anticipated that the continued improvement of analytical methods for clean-up and extraction for pesticide CSIA will prove invaluable in this application.

The application of CSIA to extensive case studies presents a significant challenge, primarily due to the presence of multiple sources of pesticides, diverse application events, and the unique characteristics of individual sites. To confront this challenge effectively, the catchment areas under investigation must be subdivided in a manner that aligns with their specific land use and water flow directions. This approach enables the utilization of a series of mixing models tailored to the respective subareas, providing a more nuanced and accurate understanding of pesticide dynamics within these complex systems (see Chap. 2). However, in the case of persistent and well-known pesticides, CSIA may offer a unique opportunity to study their long-term degradation, e.g., DDTs (Niu et al. 2016) or chlordecone. Similarly, CSIA holds substantial promise in the investigation of persistent pesticides, like atrazine, which continue to be detected in environmental field sites even decades after their prohibition in Europe (Elsner and Imfeld 2016).

Pesticide application and subsequent pollution remain a major and long-lasting environmental issue that will continue throughout the twenty-first century. The application of pesticide CSIA presents a unique opportunity to identify and quantify pesticide degradation in situ in agricultural catchments, although it requires a careful analysis of its feasibility, a robust sampling design, and a joint interpretation with hydro-climatic, geochemical, and microbial conditions and pesticide concentrations.

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Chapter 5
Stable Oxygen Isotope Composition of Phosphates to Investigate Phosphorus Cycling in the Soil–Plant Continuum

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Abstract This chapter provides an overview of how the stable isotope composition of oxygen bond to phosphorus, $\delta(^{18}\mathrm{O})_{\mathrm{PO4}}$, in phosphate can be used to investigate P cycling in the soil–plant continuum. In recent years, several books and articles about different aspects of P cycling have been published. This chapter provides summary information about P cycling in the soil–plant continuum focusing on the current methods in P research. It also provides an overview of the pitfalls of the $\delta(^{18}\mathrm{O})_{\mathrm{PO4}}$ method, especially regarding sampling and sample handling. The chapter concludes with the way forward and prospects of the $\delta(^{18}\mathrm{O})_{\mathrm{PO4}}$ method to investigate P cycling in the soil–plant continuum.

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5.1 Overview of the Phosphorus Cycling in the Soil–Plant Continuum

Phosphorus (P) is the 11th most abundant element in the Earth's crust, which is equivalent to around 0.12 %. The amount of P in the bedrocks varies (Van Kauwenbergh 2010), with the highest amounts usually found in sedimentary rocks. As bedrock is the primary source of mineral P in soils (Ruttenberg et al. 2003), the bedrock type and composition influence the total amount of P in different soil types. Total P concentrations in natural soils are usually lower than in the original bedrock material, with higher concentrations in younger than in older soils, which also differ in their relative abundance of P forms (Walker and Syers 1976). Generally, calcium-bound P dominates in younger soils, whereas occluded inorganic P and organic P forms become more abundant with progressing soil development. P concentrations and the relative proportion of various P forms vary in soils with age and have been studied extensively since the pioneering work of Walker and Syers (1976). The most recent study by Rechberger et al. (2021) examined the sorption and desorption of phosphate in volcanic soils on the Galápagos Islands. Their study sites included a climatic gradient, with annual rainfall ranging between 100 mm (dry sites) and 1600 mm (humid sites). They concluded that the main drivers for P sorption at their sites were iron (Fe) and aluminium (Al), either in amorphous phases or bound to organic matter.

The following paragraphs provide an overview of P cycling in the soil–plant continuum, for more detailed information see, for example, Arai and Sparks (2007), Raghothama and Karthikeyan (2005), White et al. (2008), Bünemann et al. (2011), Shen et al. (2011), Weihrauch and Opp (2018), and Schipanski and Bennett (2021).

5.1.1 Phosphorus Cycling in the Soil

Through weathering processes, P is released from the bedrock into the soil where it is transformed into the different organic and inorganic P forms and taken up by the soil biota. Weathering processes are divided into physical and (bio-) chemical weathering processes, and factors controlling weathering (such as climate, soil type, mineralogy, and other intrinsic soil properties) are highly inter-related (Scheffer and Schachtschabel 1998). Chemical weathering, for example through hydrolysis of minerals and oxidation of ions, of bedrock dominates in humid tropical regions, whereas physical weathering processes like thermal stress and frost weathering are more important in arid areas. The biochemical weathering of bedrock is favoured by processes such as exudation of organic acids by bacteria and plants and acidification due to the excretion of protons.

The importance of bedrock weathering for the P cycle in the soil–plant continuum is illustrated in the following paragraph. For more information see, for example, Formoso (2006), Derry (2009), Goll et al. (2014), and Ribeiro et al. (2020) and

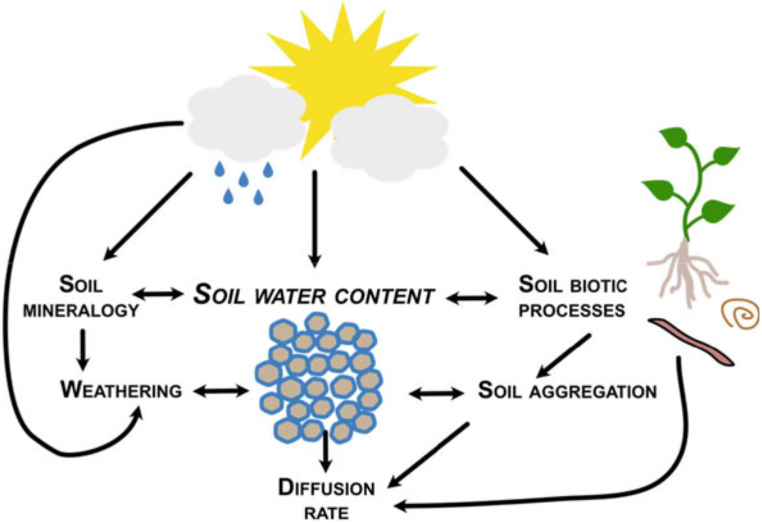

Fig. 5.1 Schematic diagram showing the complexity of soils

references within. Soil water content is used as an example in the following paragraph to illustrate the interconnectivity of the factors controlling weathering and how it influences the cycling of other P forms in the soil–plant continuum (Arai and Sparks 2007) (Fig. 5.1).

We chose the soil water content as an example as it impacts the diffusion rate of ions in the soil which in return impacts the adsorption rate of ions like phosphate (Weihrauch and Opp 2018). It also enhances the chemical weathering of bedrock material and impacts biochemical weathering, as it directly affects soil fauna and flora. Thus, soil water content is an important factor for P cycling in the soil–plant continuum. It is impacted by factors such as precipitation and temperature (climate), soil mineralogy, and soil biotic processes. Precipitation and temperature also affect soil biotic processes (Krashevska et al. 2012; Margalef et al. 2017; Nottingham et al. 2020), the P cycle (Hou et al. 2018), and influence changes in soil mineralogy (Boero et al. 1992). Through their burrowing activities, earthworms ingest soil and via this and their excretions, they also influence soil aggregation (Blouin et al. 2013), which in return influences soil microorganisms and P sorption (Linquist et al. 1997; Six et al. 2004). Earthworms, soil fauna in general, and roots create biopores through their activities, which impact soil porosity and nutrient distribution in soils (Le Bayon et al. 2021). In return, soil fauna and flora influence bedrock weathering, as well as the P cycle. Through the exudation of enzymes by soil fauna and flora, organic P is hydrolyzed and available for plant uptake.

Similarly to weathering, chemical and physical processes (including precipitation, dissolution, adsorption, desorption) as well as biological processes (including

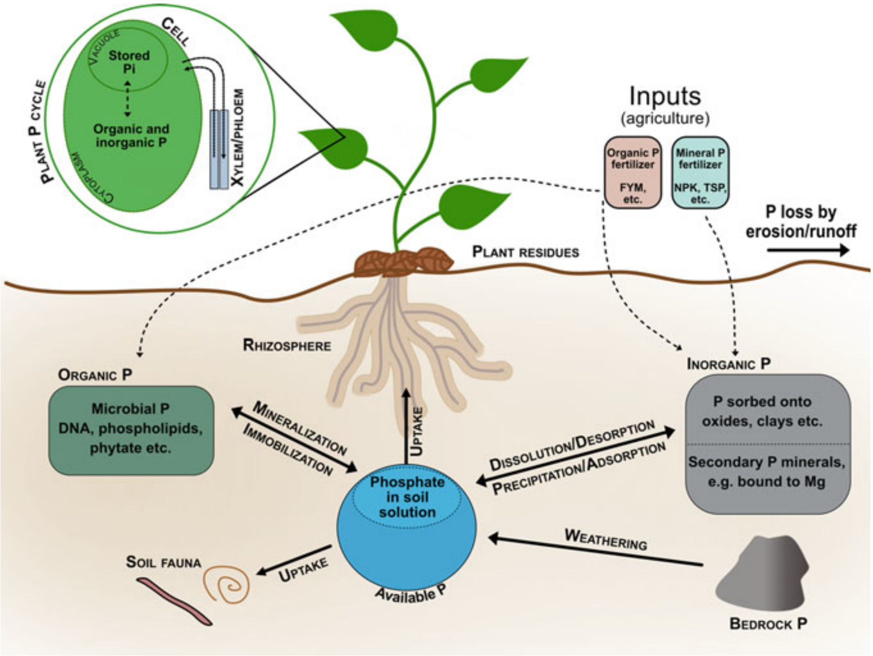

Fig. 5.2 Schematic of phosphorus (P) cycling in the soil–plant continuum

immobilization, mineralization, enzymatic hydrolysis, and the activity of soil macro-, micro-, and mesofauna and -flora) govern the P cycle in the soil–plant continuum (Fig. 5.2) (Arai and Sparks 2007; Vos et al. 2014).

Phosphorus occurs as inorganic and organic P forms (e.g. phosphate, phosphonates, and phytate) in soils, with inorganic and organic phosphates being the most abundant (McKelvie 2005; Arai and Sparks 2007; Tiessen 2008). The bioavailability of soil P varies greatly, and some P forms such as phytate are more recalcitrant than others. Only a small amount (around 0.1 mg P L^{-1}; Tiessen 2008) of inorganic P occurs as free phosphate, i.e., the readily available P, in the soil solution and can directly be taken up by plants. Phosphate in soils is most available at a neutral pH range but this range is recently under discussion (Barrow 2016; Simonsson et al. 2018). Most inorganic P occurs as phosphate bound to aluminium (Al) or iron (Fe) oxyhydroxides, to soil particles like clay minerals, and to calcium (Ca) or magnesium (Mg) (Gérard 2016). Soil microorganisms like archaea and bacteria also partly contain inorganic P (around 30 %; Bünemann et al. (2011)), but the majority of P in microorganisms occurs as organic P, such as phospholipids and nucleic acids. The relative proportion of organic P forms in microorganisms is influenced, similarly to plants (Veneklaas et al. 2012), by their P status. In P-limited aquatic bacteria, 60% of microbial P consisted, for example, of phospholipids and nucleic acids (Vadstein 1998). Microorganisms also often store P in the form of polyphosphates in their cells

(Rao et al. 2009; Akbari et al. 2021). Depending on the soil, organic P typically makes up 30 to 65 % of total soil P (Harrison 1987; Richardson et al. 2005).

5.1.2 The Fate of Fertilizer P in Soils

As concentrations of bioavailable P in agricultural soils are often insufficient to maintain crop production, farmers apply P fertilizer (Schnug and De Kok 2016; Ros et al. 2020). Phosphorus fertilizers are available as mineral and organic fertilizers. Mineral P fertilizer mainly consists of inorganic P, whereas organic P fertilizers contain inorganic P but also varying amounts of organic P compounds and other organic compounds, such as humic acids. Due to their different compositions, mineral and organic P fertilizers behave differently in soils (Audette et al. 2016) and affect P cycling in the soil–plant continuum in different ways (Keller et al. 2012; Ma et al. 2020). Adding organic P fertilizers to the soil leads to an increased input of organic matter compared to mineral P fertilizers. This can result in enhanced microbial activity in organically fertilized soils (Oehl et al. 2001). Mineral P fertilization on the other hand can lead to reduced microbial and enzymatic activity in soils (Zhang et al. 2015; Chen et al. 2019).

5.1.3 Mineral P Fertilizers

Rock phosphate, derived from P-containing ores, is the primary raw material for mineral P fertilizers, but it can also be applied directly to agricultural fields (Zapata and Roy 2004). Mineral P fertilizers are applied in agricultural fields as triple superphosphate (TSP), (mono-/di-) ammonium phosphate, combined with other nutrients such as nitrogen (N) and potassium (K), and to a lesser degree as ammonium polyphosphate, which is, unlike the first three, applied as a liquid. Only a small amount (up to 20%; Plaxton and Tran (2011)) of inorganic P derived from mineral fertilizers is taken up by plants during a growing season (Blake et al. 2000). The majority is either adsorbed by soil particles and slowly enters the more recalcitrant P pools or is lost due to leaching into the groundwater or soil erosion. In developed countries with access to fertilizers, this process translated into the build-up over a few decades of the so-called legacy P in soils (Kamprath 1967; Menezes-Blackburn et al. 2018). Ott and Rechberger (2012) estimated that in the 15 European Union Countries as of 1 May 2004 (EU-15), the net accumulation of P in agricultural soils is 2.9 kg P yr^{-1} per capita. This is approximately 62% of the net annual per capita consumption of P in the EU-15.

Organic P amendments cover a wide range of substances. Farmyard manure is probably the most common organic P amendment; other examples are biogas digestates and animal slurry. The origin of the organic P fertilizer/amendment impacts the fate of P derived from it, as shown for three different types of animal manure (Azeez and van Averbeke 2011) and a range of organic fertilizers including digestates (Vanden Nest et al. 2015). Poultry manure, for example, reduced P fixation in soils more than goat and cattle manure, perhaps caused by a competition about the sorption sites between P and humic acid (Azeez and van Averbeke 2011). Similarly, Vanden Nest et al. (2015) observed that the different compositions of organic matter in organic fertilizers lead to different effects on soil P availability. The various organic P fertilizers also contain different organic P compounds or different amounts of organic P compounds. As organic P compounds need to be hydrolyzed before they can be used by plants (see Sect. 5.3), this also impacts the availability of P derived from organic P fertilizers. Phytate is, for example, more present in poultry manure and pig slurry, as these monogastric animals lack the enzyme phytase to hydrolyze phytate (Scholz and Wellmer 2015).

5.1.4 Phosphorus and Plants

The amount of total P in soils would often be sufficient to sustain plant growth, however, only a small portion of the total soil P can readily be taken up by plants or soil microorganisms. Plants take up P as a phosphate ion, mainly as $H_2PO_4^-$ or HPO_4^{2-}, from the soil solution, creating a P concentration gradient from the rhizosphere to the bulk soil, which leads to the desorption of phosphate from soil particles and a flux of phosphate towards the roots (Hinsinger 2001; Schnepf et al. 2008; Kreuzeder et al. 2018). Since concentrations of available P in soils are usually low compared to plant P demands, plants have developed strategies to increase their P acquisition and internal P use efficiency (Table 5.1) (Hinsinger 2001; Shen et al. 2011; Veneklaas et al. 2012; Raven et al. 2018).

Acquisition strategies can roughly be divided into four categories: exudation, symbiosis, expression of high-affinity P transporters, and root architecture. Plants are known to excrete organic acids, enzymes, and protons to increase the availability of soil P. Organic acids chelate ions like Fe and thus prevent the binding of P to Fe. Pigeon pea (*Cajanus cajan*) exudes piscidic acid (p-hydroxybenzyl tartaric acid), which is known to release P bound to iron (Fe) (Ae et al. 1990; Raghothama 1999; Adu-Gyamfi 2002; Ishikawa et al. 2002; Krishnappa and Hussain 2014). Enzymes such as acid phosphatase catalyze the hydrolysis of organic P into inorganic P so that plants can take it up. As mentioned in Sect. 5.1, the soil pH affects the availability of the soil P, and therefore some plants excrete protons, which leads to an acidification of the rhizosphere. Other plants express high-affinity P transporters in the roots, which leads to an increased P flux into the roots (Rausch and Bucher 2002). Changes in root architecture can also increase P acquisition. Members of the Proteaceae species form cluster roots that excrete organic acids. The formation of proteoid (or cluster) roots is, for example, a common strategy of the Proteaceae family in Australia growing on low P soils (Lambers et al. 2015a). Among the cultivated species, white lupin (*Lupinus*

Table 5.1 Plant strategies to increase phosphorus (P) acquisition and internal P use efficiency

P acquisition	
Exudation	Organic acids
	Enzymes
	Protons (acidification of the rhizosphere)
Symbiosis	Symbiosis with mycorrhizal fungi
Expression of high-affinity P transporters in the roots	
Root architecture	Proteoid/cluster roots
	Enhanced root biomass
	Changes in root morphology and architecture (root biomass and elongation rate, number of lateral roots and root hairs, etc.)
Internal P use efficiency	
Remobilization of P stored in the vacuole	
Re-translocation of P from older to younger leaves or reproductive organs	
Replacement of phospholipids by other lipids	
Induction of acid phosphatase	

albus) is also known to form cluster roots (Lambers et al. 2015b). Phosphorus-limited plants often also invest more resources in the roots, which leads to a greater root-to-shoot ratio compared to non-limited plants. An increased number of fine roots allows plants to explore a greater soil volume. In most soils, bioavailable P concentrations are highest in the topmost soil layers. A strategy to better access this P is a change in the root architecture, with more roots growing laterally (Lynch and Brown 2001). This phenomenon is known as topsoil foraging and is increased in soybeans by a shallower root growth angle (Lynch 2011). Similarly, the symbiosis with mycorrhizal fungi greatly increases the soil volume that plants can explore and consequently have access to more bioavailable P (Schnepf et al. 2008; Smith et al. 2011). Mycorrhizal fungi, like plants and microorganisms, secrete phosphoenzymes which could increase the availability of organic P for plant nutrition (Ezawa and Saito 2018). In fact, around 90% of land plants are associated with mycorrhizal fungi (Smith and Read 2010).

Phosphorus is essential for plants as it is involved in several metabolic processes and the inorganic P concentration inside the cytoplasm is tightly regulated (Bieleski 1972; Raghothama and Karthikeyan 2005). Therefore, plants, besides their P acquisition strategies, have developed mechanisms to increase their internal P use efficiency (Plaxton and Tran 2011). More detailed information about this topic can be found in White and Hammond (2008), Rose and Wissuwa (2012), Lopez-Arredondo et al. (2014), and references therein. Inside plants, P is considered a mobile nutrient, and once it is taken up by plants, it is translocated to the different plant organs. The uptake of P and translocation to different plant organs and cell compartments involve cell membrane transporters and the xylem and phloem (Jaiwal et al. 2008; Miller et al. 2009; White 2012). Depending on the P nutrition status of a plant, most of the plant P occurs as inorganic P (Veneklaas et al. 2012) mainly stored inside the vacuole when plants are growing at a high P status (up to 85–95 %; Raghothama and Karthikeyan (2005)). The remobilization of this stored vacuolar P is one way in which plants can increase their internal P use efficiency (PUE) (Rose and Wissuwa 2012). Re-translocation of P from older to younger or reproductive organs like seeds is another strategy to increase the PUE (Akhtar et al. 2008; Richardson et al. 2011). Compared to the tightly regulated cytosolic inorganic P, the concentrations of other plant P compounds like nucleic acids, phosphorylated metabolites, and phospholipids can change more and the turnover rates of some of these P compounds are less than 1 min (Bieleski and Laties 1963; Veneklaas et al. 2012). After the addition of ^{32}P to potato plants, it took less than 1 min until half of the ATP in older potato tissue had incorporated the added label (Bieleski and Laties 1963). The replacement of phospholipids by other lipids like sulfo- and galactolipids also increases the PUE of plants (Lambers et al. 2012). Similar to increasing the P acquisition, the expression of intracellular acid phosphatases increases the PUE of plants (O'Gallagher et al. 2021).

Most modern crop varieties are less efficient in acquiring P compared to older varieties or wild types since breeding efforts focused on other traits like increased starch content or yield in general. Nowadays, researchers have investigated the P uptake and utilization efficiency of different plant varieties, and through breeding or genetically modifying crops to create new crop varieties, which have a good balance between yield and P use efficiency (Cong et al. 2020). Examples include potatoes (Balemi and Schenk 2009; Pantigoso et al. 2020), rice (Wissuwa et al. 1998; Irfan et al. 2020), and wheat (Fageria and Baligar 1999; Korkmaz et al. 2009). With these new varieties, it might be possible in future to reduce the dependence on P fertilizers, as well as reduce the amount of legacy P built up in the soil. Other research focuses on soil management strategies such as intercropping (Brooker et al. 2015; Garland et al. 2017) and microorganisms or soil meso- and macrofauna to increase P availability in the soil (Richardson et al. 2009; Blouin et al. 2013; Trap et al. 2016). Intercropping cereals with legumes can have a positive effect on the P availability of cereals (Hinsinger et al. 2011). When intercropping maize (*Zea mays*) with fava beans (*Vicia faba*), maize as well as fava bean yield increased by 43% and 26%, respectively (Li et al. 2007).

5.1.5 The Next Steps—Where Are the Biggest Gaps in Our Knowledge?

As the previous section illustrates, P cycling in the soil–plant continuum is complex and we still do not fully understand all its aspects. At the same time, dealing with P-related issues (such as declining rock phosphate reserves and pollution of aquatic systems) becomes more urgent. Several research papers recently addressed research gaps and questions regarding the P cycle in the soil–plant continuum (Yang and

Finnegan 2010; Richardson et al. 2011; Kruse et al. 2015; Reed et al. 2015; George et al. 2017). Generally, the gaps can be divided as follows:

i. **Soil intrinsic factors, management, and fauna**. Organic soil P can be an important P source for plants; however, many issues around organic P remain, for instance how to link enzymatic P cycling with operationally defined (organic) P pools or the importance of microbial mechanisms related to organic matter (George et al. 2017). The role of soil pH regarding P availability has recently been under discussion (Barrow 2016; Simonsson et al. 2018). It is also still unclear how microorganisms deal with low P conditions at acidic soil pH (Lidbury et al. 2017). Like soil pH, soil management can also affect the availability of soil P. Soil management strategies include intercropping different plant species with each other and adding specific microorganisms to the soil to enhance P availability. Despite recent advances in this field, there are still many unknowns about how soil management strategies can increase the availability of legacy P and other recalcitrant P (Menezes-Blackburn et al. 2018). Soil management also affects earthworms and other soil fauna, but the role of earthworms for the P nutrition of crops, for example, is a relatively recent field of research (Puissant et al. 2021; Trap et al. 2021).

ii. **Plant physiology and the genetics behind.** Whereas many studies, mainly under experimental conditions in the greenhouse, have examined how P starvation affects plant physiology and what genes are involved, it is still unclear what sets off the phosphate starvation response of plants (Yang and Finnegan 2010). It is also debated how relevant are data obtained in greenhouse studies for field-grown plants (Richardson et al. 2011). Greenhouse studies usually only consider a few variables like the soil P status or soil water content. In contrast, plants grown under field conditions might not only be exposed to low P concentrations but also must adapt to changes in precipitation, temperature, competition about P with microorganisms, or a heterogeneous distribution of P in the soil.

iii. **Incorporation of P into environmental models.** Improving the representation of the P cycle in models can improve the constraints of the predictions about soil C storage under climate change (Helfenstein et al. 2020). However, many model parameters regarding the P cycle are still missing (Reed et al. 2015). One aspect that is recently of high interest is linking the P to other nutrient cycles since modellers have realized the interconnectivity of nutrients (Wang et al. 2007; Reed et al. 2015; George et al. 2017; Bertrand et al. 2019). The C:N:P ratio is an important parameter for investigating the coupling of the C, N, and P cycle (Bertrand et al. 2019). Phosphorus is involved in key metabolic processes, such as providing energy for the transport of nutrients across membranes, and thus, P limitation can also affect the cycling of other nutrients. Cleveland et al. (2002) showed that in tropical forests, the utilization of labile soil organic carbon by microorganisms is P limited.

5.2 Current Approaches and Methods to Study P Cycling in the Soil–Plant Continuum

There are still many unknowns when it comes to P cycling in the soil–plant continuum. A detailed overview of current innovations regarding methods to investigate P cycling in the soil–plant continuum can be found in Bünemann et al. (2011), the IAEA TECDOC-1805 (IAEA 2016), Kruse et al. (2015), Neumann et al. (2009), and references therein. Methods to investigate P cycling in the soil–plant continuum can be divided into four groups: chemical extractions, tracers, molecular approaches, and spectroscopic methods (Bünemann et al. 2011). These methods vary in their areas of application and in the information obtained from them (Table 5.2). The chosen method therefore depends on the research questions and hypotheses. However, none of those methods should be used as a stand-alone method but should be combined with other approaches.

Recent developments in the suitability of the different methods have driven attention to approaches commonly used. Recently, the usefulness of chemical fractionations to study P in soils has been debated (Klotzbücher et al. 2019; Barrow et al. 2021; Guppy 2021; Gu and Margenot 2021). In fractionation protocols, fractions are often assigned to certain soil P pools or certain P forms are assigned to certain fractions, like iron-bound P to the NaOH fraction. However, one should be aware that, for instance, a chemically determined P pool does not necessarily adequately represent an environmentally relevant P pool. The resin-extractable P fraction or pool is for instance considered as an approximation for available P, but other fractions also might contain available P. Some methods, like the $\delta(^{18}O)_{PO4}$ technique, however, require knowledge about chemical fractionations (Tamburini et al. 2018), but the limitations of those should then be acknowledged when interpreting the results. It might be useful to combine chemical fractionations with other analytical methods, such as the determination of iron in the NaOH fraction, to validate the results obtained by chemical fractionations (Condron and Newman 2011).

The **extraction and analysis** of P fractions comprise a wide range of methods, from the routine extraction of available P in soils to the isolation of vacuolar P in plants. Most studies investigating P in the soil–plant continuum determine at least the easily extractable inorganic P (considered the available soil P) or the total P in soils. Common methods for the extraction of available P from soil samples include extraction with anion exchange resin membranes (Kouno et al. 1995), bicarbonate (Olsen 1954), water (Van der Paauw 1971), and a sodium fluoride-hydrochloric acid solution (Bray and Kurtz 1945). Not all methods are appropriate for all soil types (Oberson et al. 1997; Nawara et al. 2017; Blackwell et al. 2019), thus leading to erroneous results. Total P in soils and plant material is often determined via chemical extraction, such as acid digestion and sodium carbonate fusion (Sommers and Nelson 1972; Bowman 1988; Bender and Wood 2000; Maathuis 2013). Total P is also determined with less destructive methods like X-ray fluorescence (XRF) (see section below about spectroscopic approaches). While the extraction of available P from soils is relatively straightforward, extracting specific P compounds like phytate

Table 5.2 Common methods to investigate P cycling in the soil–plant continuum

Method		Basics	Area of application	Literature
Extraction of P forms and fractions	Specific P forms	Extraction of specific P forms like phytate and phospholipids	Phospholipids from soils as indication for microbial biomass; plant physiology (e.g. changes of P compounds in plants due to P limitation)	Bieleski and Young (1963), Ehlers et al. (2010), Tjellström et al. (2008)
	Chemical fractionation	Based on the different chemical extractability of P forms and fractions	Distribution of P among different P fractions provides rough idea about the P status of a soil	Adu-Gyamfi et al. (1990), Condron et al. (1985), Hedley et al. (1982), Levy and Schlesinger (1999), Tiessen et al. (1984)
	Specific P fractions		Extraction of available P is used in agriculture to determine the amount of P fertilizer which needs to be applied; metabolic P in plant material	Fardeau et al. (1988), Nawara et al. (2017)
Tracers	Radioisotopes of P	Application of ^{32}P or ^{33}P to soils, plants, etc.; extraction of certain P fractions and determination of radioactivity; labelling P compounds and/or fertilizers with ^{32}P or ^{33}P	Mainly used in greenhouse studies; measuring P fluxes in soils and plants; estimate gross and net organic P mineralization; uptake of P by plants and microorganisms; determination of fertilizer derived P in plants; determination of P availability and exchangeability in soils	Bünemann (2015,; Fardeau et al. (1995), Frossard et al. (2011), Helfenstein et al. (2020), Pistocchi et al. (2018), Whitfield et al. (2018)

(continued)

Table 5.2 (continued)

Method		Basics	Area of application	Literature
	Stable isotopic composition of oxygen (O) associated with P	Working with natural abundance or labelling; usually extraction of different P fractions via sequential extraction	Mainly information about biological processes in soils; tracing inorganic P input into aquatic systems	Amelung et al. (2015), Jaisi and Blake (2014), Pfahler et al. (2020b), Pistocchi et al. (2017), Tamburini et al. (2014a)
	Other tracers	In some cases, P is associated with other elements like cadmium (Cd) and uranium (U) which can be used to trace P	Used to study the fate of fertilizer P	Sun et al. (2020)
		Labelling P compounds with ^{13}C	Studying organic P compounds used by microorganisms	Gross et al. (2017)
Molecular approaches	Omics (meta-genomics, metabolomics, genomics, proteomics, transcriptomics)	Extraction of metabolites, DNA, and RNA from samples	Provides information about for example microbial community structure and composition in soils; information about proteins, metabolites, genes, etc., that are involved in a certain process	Ragot et al. (2015, 2017), Neal et al. (2017)
	Enzyme activities	Adding different substrates (depending on the targeted enzyme) and measuring, e.g. the amount of phosphate released with time	Potential enzyme activities in soils; common examples include acid and alkaline phosphatase, phytase	Araújo et al. (2008), Louche et al. (2010), Nannipieri (2011), Tabatabai and Bremner (1969)

(continued)

Table 5.2 (continued)

Method		Basics	Area of application	Literature
Spectroscopic and spectrometric approaches	^{31}P-NMR spectroscopy	Solid or liquid state spectroscopy, liquid state spectroscopy requires extraction prior to analysis	Used to analyse P-containing compounds, especially organic P in soils	Annaheim et al. (2015), Cade-Menun and Liu (2014), McLaren et al. (2015), Turner et al. (2012), Vincent et al. (2010)
	X-ray absorption spectroscopy	Semi-quantitative analysis based on spectral features of different P species	Mainly used for investigating mineral P forms in soils; potentially useful also for investigating organic P	Negassa and Leinweber (2009), Schmieder et al. (2020)
	NanoSIMS		For example, localization of P in cells	Gross et al. (2020), Kilburn et al. (2010), Werner et al. (2017)

from soil and plant material is often more laborious (Frank 2013; Reusser et al. 2020; Turner et al. 2020). To quantitatively analyse phytate in soil and plant material, the samples were first extracted with an acid or alkaline solution, followed by isolating phytate from the extract. Methods for isolation include high-performance liquid chromatography (HPLC) and precipitating phytate as an insoluble salt, for example, by adding iron (Frank 2013; Turner 2020).

Using tracers to investigate nutrient cycling is a common research approach, and in the case of P, it includes the two radioisotopes ^{32}P and ^{33}P, the stable isotope composition of oxygen (O) associated with P, ^{13}C-labelled organic P compounds, and using elements that are associated with P, such as uranium (U) in the case of fertilizers. The application areas of P radioisotopes are quite diverse (Frossard et al. 2011) and include determining the residence time of P in different soil P pools (Helfenstein et al. 2020) and tracing P inside plants once it has been taken up (Mimura et al. 1996). Recently, Whitfield et al. (2018) published a method to synthesize inositol hexakisphosphate (IHP) labelled with ^{32}P, which could advance our understanding of IHP cycling in soils.

Using molecular approaches is becoming increasingly accessible. While only a few publications used omics analysis to investigate P cycling in the soil–plant continuum back in 2011 (Wasaki and Maruyama 2011), those methods are nowadays more common and they brought useful insights, for example, into the phosphate starvation response (PSR) of plants (Lopez-Arredondo et al. 2014; Lan et al. 2017). Proteomics revealed that the PSR of plants also affects the enzymes involved in the tricarboxylic acid cycle of plants (Lan et al. 2017).

Spectroscopic and spectrometric approaches cover a wide range of techniques (Kruse et al. 2015). Determining total P in soils via XRF instead of chemical extraction often yields more accurate results, as chemical extractions tend to underestimate the concentrations of total soil P (Chander et al. 2008; Wang et al. 2021b). The advantages and disadvantages of ^{31}P-NMR are an ongoing debate. One disadvantage is that some organic P compounds might be hydrolyzed prior to or during NMR analysis. By using ^{18}O-enriched medium this issue could potentially be solved (Wang et al. 2021a). Combining ^{31}P-NMR with an ^{18}O-label is also used to study enzymatic mechanisms (Cohn 1958; Cohn and Hu 1978). Spectroscopic approaches also include other approaches that are currently less common than those mentioned in Table 5.1 (Kruse et al. 2015). One example is nanoscale secondary ion mass spectrometry (NanoSIMS). Rodionov et al. (2020) used NanoSIMS in combination with other techniques to study P cycling in forest subsoils by visualizing the distribution of P in the rhizosphere.

5.3 The $\delta(^{18}O)_{PO4}$ to Investigate P Cycling in the Soil–Plant Continuum—Current State

5.3.1 Background of the Method

In recent years, using the stable isotope composition of oxygen (O) associated with P [$\delta(^{18}O)_{PO4}$] to study phosphorus (P) cycling in the soil–plant continuum has become more common. Detailed reviews about the principles of the method and its applications can be found in Adu-Gyamfi and Pfahler (2022), Bauke (2021), Tamburini et al. (2014b), and Jaisi and Blake (2014). In short, the principles of this method are (1) in the environment, P is mainly associated with O and (2) the P-O bond is stable under the absence of biotic processes and at the Earth's surface conditions (Winter et al. 1940). Alterations of $\delta(^{18}O)_{PO4}$ values in the environment occur due to processes that lead to cleaving of the P-O bond (mainly biotic processes) or sorting of the heavier and lighter isotopologues (biotic and abiotic processes) (Adu-Gyamfi and Pfahler 2022). Biotic processes include enzymatic activity, such as organic P mineralization through enzymes and P uptake by microorganisms. One of the most important enzymes influencing $\delta(^{18}O)_{PO4}$ values is inorganic pyrophosphatase (PPase). This ubiquitous enzyme leads to the progressive exchange of all four O atoms in phosphate with O from surrounding water and causes a temperature-dependent isotopic equilibrium between O in phosphate and in water (Cohn 1958; von Sperber et al. 2017). Equilibrium $\delta(^{18}O)_{PO4}$ values can be calculated (Chang and Blake 2015) and are often used as an indicator for microbial P cycling, since the PPase is an intracellular enzyme (Tamburini et al. 2012). Other enzymes, phosphomono- and -diesterases such as acid and alkaline phosphatases among others, lead to an exchange of one to two O atoms between the phosphate moiety and water (Liang and Blake 2006a; von Sperber et al. 2014; Wu et al. 2015). The reported fractionation factors are mostly negative; i.e., phosphate released from organic P is usually depleted in ^{18}O compared to the original organic P compound. Acid phosphatase for example has a fractionation factor of approximately -10 ‰ (von Sperber et al. 2014). With a $\delta(^{18}O)_{H2O}$ value of the surrounding water of around 0 ‰ and organic P with a $\delta(^{18}O)_{PO4}$ value of around 20 ‰, phosphate released from organic P via acid phosphatase would have a $\delta(^{18}O)_{PO4}$ value of 10 ‰. Abiotic processes like the interaction of phosphate with iron oxides have a rather small fractionation factor compared to biotic processes (Jaisi et al. 2010; Melby et al. 2013a).

To determine the $\delta(^{18}O)_{PO4}$ of a P fraction, the targeted fraction needs to be extracted from the environmental sample, e.g. soil, and afterwards isolated to precipitate the final analyte silver phosphate. Nowadays, protocols for the extraction, isolation, and purification of phosphate for determination of $\delta(^{18}O)_{PO4}$ values exist for organic and inorganic P forms and fractions in soils, plants, sediments, fertilizers, and water (see Adu-Gyamfi and Pfahler, 2022 for an overview). Most protocols consist of a stepwise purification of an extract/sample (McLaughlin et al. 2004; Tamburini et al. 2010, 2018). The most common protocol for soils and plants consists of four steps: (1) precipitation of ammonium phosphomolybdate (APM), (2) dissolution of APM and precipitation of magnesium ammonium phosphate (MAP), (3) dissolution of MAP and addition of exchange resin to remove cations, (4) removal of resin and addition of silver ammine solution to precipitate silver phosphate. For a more detailed description of the method see also IAEA book, Tamburini et al. (2010), and Tamburini et al. (2018).

5.3.2 *Calculations*

To assure the quality of the obtained isotope data, the following calculations should be performed prior to further data analysis:

- Estimation whether or not **inorganic hydrolysis** of organic or precipitated P occurred during the extraction of a P pool (McLaughlin et al. 2006; Pistocchi et al. 2017).
- **Recovery of a label** like ^{18}O-labelled phosphate or water added to soils, plants, or nutrient solutions (Gross and Angert 2015; Bauke et al. 2021).

To interpret the obtained isotope data, the following calculations are useful:

- **Isotopic equilibrium** between O in phosphate and in water caused by the PPase (Chang and Blake 2015).
- Effect of **organic P hydrolysis** via phosphomono- and -diesterases on $\delta(^{18}O)_{PO4}$ values (see Table 5.1 under “Enzymes”).

The calculated isotopic equilibrium and the effect of organic P hydrolysis can then, for instance, be used in a mass balance to estimate the relative contribution of microbial P cycling and enzymatic hydrolysis of organic P to available soil P. **Mass balances** can also be used to estimate the contribution of different P pools, such as mineral P and inorganic P in plants to available soil P (Tamburini et al. 2012) (see also Chap. 3).

5.3.3 Application of the Method to Study Phosphorus Cycling in the Soil–Plant Continuum

With more protocols available for different P pools and environmental samples, the $\delta(^{18}\text{O})_{\text{PO4}}$ method is currently used to investigate a wider range of aspects of the P cycle in the soil–plant continuum (Table 5.3). See also Bauke (2021) for a more detailed discussion about soil $\delta(^{18}\text{O})_{\text{PO4}}$ values.

Table 5.3 Aspects of the phosphorus (P) cycle in the soil–plant continuum to which the $\delta(^{18}\text{O})_{\text{PO4}}$ method has been applied so far

Aspect of phosphorus cycle		References
Enzymes	Acid phosphatase	von Sperber et al. (2014)
	Alkaline phosphatase	Blake et al. (2005)
	5’-nucleotidase	Liang and Blake (2006b)
	Phytase	von Sperber et al. (2015), Sun et al. (2017)
	Inorganic pyrophosphatase	Blake et al. (2005), Chang and Blake (2015), von Sperber et al. (2017)
	PDase1	Liang and Blake (2009)
	DNase	
Soil processes/ properties	Adsorption/ desorption	Jaisi (2013), Jaisi et al. (2010), Melby et al. (2013a)
	Organic acids	Wang et al. (2016)
	Soil pH	Pfahler et al. (2020b)
	Nitrogen	Bauke et al. (2018), Bi et al. (2018), Frkova et al. (2021), Jing et al. (2021), Shen et al. (2020), Wang et al. (2021b), Weiner et al. (2018)
	Carbon	Wang et al. (2021b)
	Biopores	Bauke et al. (2017)
	Soil development	Helfenstein et al. (2018), Roberts et al. (2015), Tamburini et al. (2012)
	Microbial P cycling	Pistocchi et al. (2020), Siegenthaler et al. (2020), Tamburini et al. (2012)
Plants	P limitation	Pfahler et al. (2017, 2020a, b)
	P uptake	Bauke et al. (2021)
Fertilizers		Smith et al. (2021), Sun et al. (2020)

One of the first studies which used ^{18}O in phosphate to study P cycling in soils was the study by Larsen et al. (1989). They added KH_2PO_4 labelled with ^{18}O and ^{32}P to soil, distributed the soil among different pots, and grew grass in those pots for three months. To exclude biotic activity, they also added germicide to some pots for three months. Half of the added ^{18}O-label was lost in the untreated soils, whereas no loss was observed in the germicide-treated soil, which was attributed to a lack of biotic activity (for more information about labelling experiments see also Chap. 3). In line with this, Tamburini et al. (2012) showed by analysing $\delta(^{18}O)_{PO4}$ values of soil and plant P pools along a soil chronosequence that prior to being released to the available soil P pool, phosphate is cycled through microbes. In an incubation experiment with organic horizons of two forest soils, differing in their amount of available P, Pistocchi et al. (2020) added ^{18}O-labelled water to the soils. In the soil with a high P availability, $\delta(^{18}O)_{PO4}$ values of available P approached equilibrium values, whereas in the low-P soil, the impact of hydrolyzing enzymes was visible in $\delta(^{18}O)_{PO4}$ values of available P, thus showing that this pool was mostly replenished by P derived from organic P mineralization.

Whereas more and more studies use $\delta(^{18}O)_{PO4}$ values to investigate P cycling in soils, less is known about plants, i.e. P acquisition by plants and plant internal P cycling, and how these processes affect $\delta(^{18}O)_{PO4}$ values. Plants play a vital role for P cycling in soils, as they not only take up P from the soil solution, but also exude enzymes and create biopores thereby altering chemical and physical soil properties. Plant litter can also be a major source of P in soils (Sayer et al. 2020); therefore, it is necessary to understand how plants, under different environmental conditions, alter $\delta(^{18}O)_{PO4}$ values. In a greenhouse experiment with soybeans, Pfahler et al. (2013) showed that $\delta(^{18}O)_{PO4}$ values of inorganic P, extracted from plant leaves, are more enriched in ^{18}O compared to P supplied in the nutrient solution. This was most likely caused by the O exchange between phosphate and leaf water, which is usually enriched in ^{18}O compared to soil water, due to transpiration. In a follow-up study, Pfahler et al. (2017) investigated if $\delta(^{18}O)_{PO4}$ values can be used to study plant response to P limitation. Combining $\delta(^{18}O)_{PO4}$ values with radioactive ^{33}P labelling, they showed that $\delta(^{18}O)_{PO4}$ values of trichloroacetic acid (TCA)-extractable P from soybean leaves, subjected to P limitation, are less enriched in ^{18}O compared to the soybeans growing with an ample amount of P in the nutrient solution. This was most likely caused by hydrolyzing enzymes like acid phosphatase, releasing, and then translocating P from senescent to younger leaves. Recently, several studies have investigated whether the $\delta(^{18}O)_{PO4}$ method can be used to trace P inside plants after uptake (Qin et al. 2018; Hauenstein et al. 2020; Bauke et al. 2021). Qin et al. (2018) used ^{18}O-labelled phosphate to trace applied phosphate from the soil to maize shoots. They found this possible only when the roots of maize plants were not inoculated with an arbuscular mycorrhizal fungus (AMF). They concluded that the ^{18}O-label was lost during phosphate metabolism inside the AMF (Qin et al. 2018). Hauenstein et al. (2020) and Bauke et al. (2021) used ^{18}O-labelled water instead of ^{18}O-labelled phosphate to investigate P cycling in plants. The ^{18}O-labelled water was provided to the plants as irrigation water or in the nutrient solution. Hauenstein et al. (2020) used this method to study P nutrition of beech at two forest fertilization experiments in Germany. Based on the analysis of $\delta(^{18}O)_{PO4}$ values of inorganic P in the xylem sap, they postulate that in the P fertilized treatments, the additional P in the xylem was derived from fertilizer P and biologically cycled soil P. Bauke et al. (2021) used spring wheat as a model plant and conducted three different experiments using either hydroponic systems or pots filled with soil. Unlike shoots, root $\delta(^{18}O)_{PO4}$ values of TCA P partly preserved the $\delta(^{18}O)_{PO4}$ value of the P source due to lower metabolic activity in the roots. They therefore suggested that root P might provide better information on the P source than above-ground biomass, although their study did not consider the effects of mycorrhization, as in Qin et al. (2018).

As shown in the above-mentioned studies, plants can greatly alter $\delta(^{18}O)_{PO4}$ values of assimilated phosphate. Hacker et al. (2019) showed that plant diversity, via its effect on evaporation and thus soil water $\delta(^{18}O)_{H2O}$ values, can also indirectly impact $\delta(^{18}O)_{PO4}$ values of bioavailable soil P. To show this, two waters, differing in their $\delta(^{18}O)_{PO4}$ values, were applied to 27 plots in the Jena experiment, which consisted of around 80 plots with different plant species combinations. Microbial P turnover influenced $\delta(^{18}O)_{PO4}$ values of bioavailable soil P more in plots with high plant diversity compared to plots with lower plant diversity (Hacker et al. 2019).

Plants can potentially affect soil $\delta(^{18}O)_{PO4}$ values in many ways, not just via their impact on evaporation. Plants, like microorganisms, exude enzymes that are known to alter $\delta(^{18}O)_{PO4}$ values (see Table 5.1). However, little is known about how other plant acquisition strategies, such as symbiosis with AMF might affect soil $\delta(^{18}O)_{PO4}$ values. To the best of our knowledge, only Wang et al. (2016) investigated the impact of low-molecular-weight organic acids (LMWOAs) on $\delta(^{18}O)_{PO4}$ values. They used hydroxyapatite and different LMWOAs (acetic, oxalic, and citric acid) in batch and column experiments. The observed fractionation factors were relatively small: −0.3 to 1.1 ‰ (batch experiment) and −1.3 to 1.1 ‰ (column experiment).

5.3.4 Natural Abundance Versus Labelling Experiments

Most field studies work with the natural abundance of ^{18}O in the environment. However, the differences between $\delta(^{18}O)_{PO4}$ values of P pools between treatments or between different P pools are sometimes within 1 or 2 ‰ and it might be difficult to draw sound conclusions, especially when replication is missing (Pfahler et al. 2020b). One possibility is to work with strong environmental gradients, as in the pioneering field study by Tamburini et al. (2012) at a soil chronosequence. In other cases, the addition of an ^{18}O-labelled P source or ^{18}O-labelled water is advisable. Labelling experiments have great potential to bring further insights into the P cycle in the soil–plant continuum. Following the work by Larsen et al. (1989) in soils, Melby and co-authors conducted several studies using ^{18}O-labelled phosphate (Melby et al. 2011, 2013a, b). In the first study, Melby et al. (2011) described a method how to produce ^{18}O-labelled phosphate to be used in other studies. They then conducted two studies in which they used ^{18}O-labelled phosphate to investigate biological P cycling (Melby et al. 2013b) and phosphate sorption (Melby et al. 2013a). In recent years, more and more studies, including the ones mentioned before, have applied an ^{18}O-label to soils/plants, either via phosphate or via water. Most of them are conducted under controlled environmental conditions (i.e., greenhouse or growth chambers), since the application of a labelled compound in the open environment requires more care and might not always be suitable. In areas with high precipitation like tropical rainforests or high evaporation like deserts, ^{18}O-labelled water applied to the soil might rapidly be altered, which needs to be accounted for when interpreting the obtained data (Kendall and McDonnell 2012; Beyer and Penna 2021). In addition to adding a ^{18}O-labelled compound, also the addition of other labels like ^{33}P/^{32}P (Pfahler et al. 2017; Siegenthaler et al. 2020) and ^{13}C can bring useful insights into P cycling (Gross and Angert 2017). In an incubation study, Siegenthaler et al. (2020) applied ^{32}P as well as ^{18}O-labelled water to soil samples from a climatic gradient in Hawaii. They found that both techniques complement each other like in case of NaOH-EDTA P where ^{32}P and $\delta(^{18}\text{O})_{\text{PO4}}$ values both indicated that NaOH-EDTA P was only partly cycled during the incubation experiment. Combining $\delta(^{18}\text{O})_{\text{PO4}}$ with labelling an organic P compound with ^{13}C, Gross and Angert (2017) showed that this combination could help elucidating both, C and P, cycling in the environment.

5.4 Challenges of the $\delta(^{18}\text{O})_{\text{PO4}}$ Method Application

The major pitfalls of the $\delta(^{18}\text{O})_{\text{PO4}}$ method can be grouped into three categories: sample handling mistakes, issues during purification, and lack of additional data for results interpretation.

5.4.1 Sample Handling Mistakes

$\delta(^{18}\text{O})_{\text{PO4}}$ is strongly influenced by biotic processes. It is therefore essential to reduce biological activity immediately after sampling to not alter the $\delta(^{18}\text{O})_{\text{PO4}}$ of any targeted P pool or compound. Drying soil samples might be effective in reducing biological activity, but drying and re-wetting are known to lyse microbial cells and release microbial P into the available P pool (Dinh et al. 2017). Thus, analysing $\delta(^{18}\text{O})_{\text{PO4}}$ of available P extracted from dried soils is different from $\delta(^{18}\text{O})_{\text{PO4}}$ values determined using fresh soils. A relatively simple way to reduce biological activity is to store the samples on ice in a freezer/polystyrene box. However, prolonged storage of fresh soil samples, even at 4 °C, might affect $\delta(^{18}\text{O})_{\text{PO4}}$ values, especially those of more labile P pools. Storing fresh soil samples for a minimum amount of time, even in the fridge, is thus recommended.

5.4.2 Extraction Issues

One issue when working with plants and $\delta(^{18}O)_{PO4}$ is that enzymatic activity needs to be stopped during extraction, otherwise $\delta(^{18}O)_{PO4}$ could be altered (Bieleski 1964). The combination of low temperatures and trichloroacetic acid was relatively efficient in stopping enzymes during extractions. Similarly, resin and microbial P extractions for $\delta(^{18}O)_{PO4}$ analysis are usually conducted at 4 °C (Tamburini et al. 2012).

Another issue associated with extracting P for $\delta(^{18}O)_{PO4}$ analysis from soil samples is that sometimes large soil volumes are required to obtain a sufficient amount of P (10–20 μmol P) for the purification protocol (Pfahler et al. 2020a). This is challenging for two reasons: (1) keeping the original soil-to-solution ratio for an extraction might be difficult and (2) reducing large volumes of extracts is laborious and needs to be done carefully (Adu-Gyamfi and Pfahler 2022). Keeping the original soil-to-solution ratio is important, as changes to it might result in extracting more or less P compared to the original ratio (Weiner et al. 2011; Pfahler et al. 2020a) and thus comparing results between studies is difficult. Large volumes of extracts need to be concentrated, for instance via brucite precipitation to approximately 100 mL to purify it and precipitate silver phosphate as the final analyte. Several brucite precipitations might be necessary, which could result in a loss of P if not done carefully (Adu-Gyamfi and Pfahler 2022).

5.4.3 Issues During the Purification Protocol

Adu-Gyamfi and Pfahler (2022) addressed the issues during the purification protocol in detail. In brief, issues during the purification of extracts are often due to high quantities of organic matter in the extracts or low P concentrations. There are ways to deal with those issues, but one must be aware of them first. An additional step with a DAX resin or (several) brucite precipitations could be included to remove more organic matter (Adu-Gyamfi and Pfahler 2021). Chemicals used in the purification protocol could potentially contain small impurities of P. Also, some detergents used in laboratories to clean equipment contain P. Thoroughly checking for P contaminations is therefore a good practice, especially when handling samples with low P concentrations.

Soils and soil extracts rich in calcium, iron, and silica might cause problems during the purification protocol (Adu-Gyamfi and Pfahler 2022; Tamburini et al. 2018). Knowledge of the chemistry of the studied soils is thus recommended to be able to prevent and face these problems.

5.4.4 Pitfalls in ^{18}O-labelling Experiments

In addition to the above-mentioned pitfalls, there are additional ones when setting up labelling experiments with ^{18}O-labelled water or a ^{18}O-labelled P compound/pool. One pitfall might be the sensitivity and linearity of isotope ratio mass spectrometry (IRMS) measurements, as observed in the case of other isotope studies (Blessing et al. 2008). Measuring ^{18}O-labelled and unlabelled samples in separate IRMS runs is advisable to avoid carry-over memory effects. Most available silver phosphate reference materials (store-bought) do not cover a wide range of $\delta(^{18}\mathrm{O})_{\mathrm{PO4}}$ values. Typically, they range between 14 and 23 ‰. It might therefore be necessary to produce an in-house standard (Lecuyer et al. 1999), covering the expected range of $\delta(^{18}\mathrm{O})_{\mathrm{PO4}}$ in the experiment (Halas et al. 2011; Watzinger et al. 2021).

Like for other labelling experiments, background information of the soil- or plant-inherent P concentration and isotope values is necessary to allow choosing an adequate labelling strength. Especially in samples with high P concentrations, the added label may be strongly diluted, and the intended processes will not become apparent. It is recommended to perform a preliminary test consisting of four steps: (1) analysis of $\delta(^{18}\mathrm{O})_{\mathrm{H2O}}$ of soil water, (2) analysis of $\delta(^{18}\mathrm{O})_{\mathrm{PO4}}$ in different soil P pools, (3) calculate theoretical equilibrium $\delta(^{18}\mathrm{O})_{\mathrm{PO4}}$ values using different values for δ^{18}O of soil water, and (4) choose a $\delta(^{18}\mathrm{O})_{\mathrm{H2O}}$ of soil water which would result in a well distinct, for instance, by a factor of 5 or 6, $\delta(^{18}\mathrm{O})_{\mathrm{PO4}}$ equilibrium value. For label application, a homogenous distribution of the labelled substance (water or P compound/pool) to a soil (in the field or pot) is crucial for ^{18}O-labelling experiments. Sample homogeneity could also be an issue when using ^{18}O-labelled water or P compounds. During the experiment and subsequent purification or sample treatment procedures, labelled samples should also be handled with care and contact with unlabelled samples should be avoided to avoid any carry-over of the label.

5.4.5 Lack of Additional Information

Phosphate mainly exchanges O with water, and $\delta(^{18}\mathrm{O})_{\mathrm{H2O}}$ of water plays an important role in interpreting $\delta(^{18}\mathrm{O})_{\mathrm{PO4}}$ values. Analysing $\boldsymbol{\delta(^{18}\mathrm{O})_{\mathrm{H2O}}}$ of water in soils and plants requires the extraction of the water, usually via cryogenic distillation. For more information about cryogenic distillation and discussions about it see, for example, Orlowski et al. (2016) and references therein. Evapotranspiration influences $\delta(^{18}\mathrm{O})_{\mathrm{H2O}}$ of soil and plant water. Putting sub-samples of the soil and plant samples into appropriate containers directly at the time of sampling is therefore recommended. Because the equilibrium promoted by PPase is temperature-dependent, measuring the ambient/soil/leaf temperature is highly recommended. One of the major aspects of P cycling in the soil–plant continuum is the P availability. Determining at least the concentrations of available P (soils), total P (soils, plants), and inorganic P (plants) is thus recommended. Knowledge of other soil properties, such as soil pH, organic matter content, and bulk density, might also help in interpreting $\delta(^{18}\mathrm{O})_{\mathrm{PO4}}$ values.

5.5 Way Forward and Prospects of the $\delta(^{18}\mathrm{O})_{\mathrm{PO4}}$ Method for Plant P Cycling

5.5.1 Earth System Models and $\delta(^{18}\mathrm{O})_{\mathrm{PO4}}$

Recently, P has been incorporated into Earth system models in addition to carbon (C) and nitrogen (N) (Reed et al. 2015). Advances in those models could be made if we have a better understanding of P mineralization, how P could limit C cycle processes, P sorption, and the coupling between P, C, and N cycling (Reed et al. 2015). $\delta(^{18}\mathrm{O})_{\mathrm{PO4}}$ has the potential to advance our understanding in these aspects, especially when biotic processes are involved. Studies conducted so far indicate that $\delta(^{18}\mathrm{O})_{\mathrm{PO4}}$ could provide useful information about the role of soil pH, enzymes, and soil microorganisms for P cycling in the soil–plant continuum, the effect of P limitation, the interaction of P with other nutrients such as N, and the residence times of P in different soil P pools (see Sect. 5.3). The first attempts to use $\delta(^{18}\mathrm{O})_{\mathrm{PO4}}$ for modelling/estimating P fluxes exist (Tamburini et al. 2012; Jaisi et al. 2017; Pfahler et al. 2017; Helfenstein et al. 2018; Bauke et al. 2021), but further research is needed.

5.5.2 $\delta(^{18}\mathrm{O})_{\mathrm{PO4}}$ Values of Single Organic Phosphorus Compounds

To advance Earth system models even further, knowing $\delta(^{18}\mathrm{O})_{\mathrm{PO4}}$ values of specific organic P compounds might be useful. Knowing those values, one could more accurately estimate the parameters associated with P mineralization. The determination of $\delta(^{18}\mathrm{O})_{\mathrm{PO4}}$ of organic P usually requires treating the pure organic P compound with UV light to hydrolyze the organic P and release inorganic P (Blake 2005; Tamburini et al. 2018). Wu et al. (2015) and Sun et al. (2017) determined $\delta(^{18}\mathrm{O})_{\mathrm{PO4}}$ of phytate via direct pyrolysis in the TC/EA-IRMS. So far, studies which analysed $\delta(^{18}\mathrm{O})_{\mathrm{PO4}}$ values of single organic P compounds have always used pure compounds and did not extract them from environmental samples. The next step might be to investigate whether some of the available methods for extracting single organic P compounds from environmental samples are suitable for the determination of $\delta(^{18}\mathrm{O})_{\mathrm{PO4}}$ values. Phytate can be isolated from soil samples via hypobromite oxidation (Turner 2020). However, due to the experimental conditions, it is necessary first to adapt this method to the phosphate purification protocols currently available, so that reliable soil phytate $\delta(^{18}\mathrm{O})_{\mathrm{PO4}}$ values can be obtained.

5.5.3 More Laboratory and Glasshouse Studies Needed to Advance the Application of $\delta(^{18}O)_{PO4}$ in the Field

So far $\delta(^{18}\mathrm{O})_{\mathrm{PO4}}$ studies have focused on hydrolyzing enzymes but data on the fractionation factors of synthesizing enzymes and phosphotransferases, to name a few, for the $\delta(^{18}\mathrm{O})_{\mathrm{PO4}}$ are missing. Some papers, however, report isotopic effects of $^{18}\mathrm{O}$ on the rate constants of these enzymes, for example Hengge et al. (2002). Information about those enzymes is vital, especially for the P cycle in microorganisms and plants. How microorganisms deal with low soil pH and low soil P conditions simultaneously is also not well understood (Lidbury et al. 2017). The $\delta(^{18}\mathrm{O})_{\mathrm{PO4}}$ might help shed light on this, as indicated in the study of Pfahler et al. (2020b). Glasshouse studies excluding variables such as changes in the vegetation but focusing mainly on the soil pH might therefore be useful. As mentioned by Reed et al. (2015), better knowledge about P acquisition strategies is necessary to improve models. Excreting organic acids is one P acquisition strategy, and one study showed that the dissolution of hydroxyapatite by organic acids (oxalic, citric, and acetic acid) leads to a small isotopic fractionation (Wang et al. 2016). As organic acids mainly affect processes in the rhizosphere, studies determining $\delta(^{18}\mathrm{O})_{\mathrm{PO4}}$ values in the rhizosphere are encouraged. This is challenging due to the small amounts of rhizosphere soil that can be collected. Rodionov et al. (2020) found no differences in $\delta(^{18}\mathrm{O})_{\mathrm{PO4}}$ values of HCl-extractable P between bulk and rhizosphere soil samples. $\delta(^{18}\mathrm{O})_{\mathrm{PO4}}$ values of the more actively cycled P pools, such as microbial and available P, extracted from rhizosphere samples have not yet been reported.

5.5.4 Lack of Field Studies Including Plants and Ecosystems

In general, information about $\delta(^{18}\mathrm{O})_{\mathrm{PO4}}$ of plants is still limited (see Sect. 5.3). Detailed plant studies are thus far only available for soybeans (Pfahler et al. 2013, 2017), maize (Qin et al. 2018), and spring wheat (Bauke et al. 2021). Additionally, Tamburini et al. (2012) analysed different plant species along a soil chronosequence at the Damma Glacier in Switzerland, Helfenstein et al. (2018) reported $\delta(^{18}\mathrm{O})_{\mathrm{PO4}}$ values for different plant species at a climatic gradient in Hawaii, and Pfahler et al. (2020b) determined $\delta(^{18}\mathrm{O})_{\mathrm{PO4}}$ of bulk vegetation samples from a grassland experiment in the UK. Those studies provided useful hints/insights about using $\delta(^{18}\mathrm{O})_{\mathrm{PO4}}$ in plant studies, but data is still limited. The effect of P limitation has so far only been investigated in soybeans, and the study by Pfahler et al. (2020b) indicates that P limitation has a similar effect on grasslands. Studies about the effect of P limitation on major crops, such as maize, rice, and wheat, especially under field conditions, are still missing. Likewise, there are no studies using $\delta(^{18}\mathrm{O})_{\mathrm{PO4}}$ and investigating the effect of crop rotation or intercropping on P cycling. Crop rotations or intercropping often combine plants with different P acquisition strategies to increase the P use efficiency of crops, and it could be expected to have an influence on the $\delta(^{18}\mathrm{O})_{\mathrm{PO4}}$ of soil and plants.

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Chapter 6
Stable Sulfur and Oxygen Isotope Compositions of Sulfates to Disentangle Agrocontaminants from Other Sources of Sulfur in Agrosystems

G. Skrzypek

Abstract This chapter summarizes key information about the sulfur cycle and stable sulfur isotope biogeochemistry. The overview includes a description of the range of the stable sulfur isotope compositions in natural environments, potential pollutants and fertilizers used in agriculture. The primary focus is on the generation, transport, and mixing of sulfate and on its stable sulfur and oxygen isotope compositions that can be used as tracers. Several case studies that apply stable sulfate isotope analyses for the identification and quantification of pollutants in natural environments are used to provide an overview of successful applications and highlight major challenges. These are followed by the principles of sulfate sampling and analytical techniques.

6.1 Overview of Stable Sulfur Isotope Biogeochemistry

Sulfur, as one of the macro-elements (H, C, N, O and S) is required for the biosynthesis of methionine and cysteine, two canonical amino acids that are incorporated into proteins (Brosnan and Brosnan 2006) and therefore is essential for the biosphere. Sulfur exists in natural environments at several oxidation states, but the most common are −2, +2, +4, and +6. The highest oxidation state (+6) is the most important for sulfur assimilation by plants because sulfur is mainly taken up as the sulfate ion, SO_4^{2-} (Davidian and Kopriva 2010). Sulfate is also an abundant ion (~2.7 g/L) in marine environments (Andrulionis and Zavialov 2019), but some terrestrial ecosystems can be sulfur limited, despite a highly dynamic sulfur cycle and significant exchanges between land and sea (Fig. 6.1). The sulfate budget of the global ocean is controlled by: (1) river discharge (Burke et al. 2018); (2) sulfate reduction and sulfide re-oxidation; and (3) burial of sulfur-bearing minerals (Jørgensen et al. 2019). The terrestrial sulfur budget reflects primary inputs from (1) oceans via the atmosphere

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as inorganic sulfate and biogenic origin dimethylsulfide (Alon et al. 2013); (2) rock erosion resulting in sulfate dissolution and sulfide oxidation (Szynkiewicz et al. 2015b); and (3) organic matter decomposition (Schroth et al. 2007; Joo et al. 2022). In addition to these natural sources, the sulfur pool in agrosystems is supplemented by various anthropogenic sources, such as: (1) industrial outputs (Górka et al. 2017); (2) mining (Dogramaci et al. 2017); and (3) agriculture pollution (Chalk et al. 2017). Therefore, the use of chemical concentrations alone does not allow partitioning of sulfur sources or assessment of pollution inputs. This challenge can be addressed by including stable sulfur isotope analyses in hydrochemical analyses, as the stable sulfur isotope composition provides fingerprints of the original S-sources or leading processes and allows calculation of the proportional contribution of these sources.

Sulfur has four stable isotopes with a broad range of natural abundances: $^{32}S = 95.04\ \%$, $^{33}S = 0.75\ \%$, $^{34}S = 4.20\ \%$ and $^{36}S = 0.01\ \%$ as mole fraction percentages, and its stable isotope composition widely varies across different environments (Coplen et al. 2002a). Because the majority of the stable sulfur isotope fractionations are mass dependent, the ratio between the two most abundant stable isotopes $^{34}S/^{32}S$ is measured primarily using isotope ratio mass spectrometry (IRMS) (Sharp 2017). The results are usually reported in permilles (‰) as 1000 isotope delta values $\delta(^{34}S)$ with respect to the absolute abundance in the Cañon Diablo Troilite (VCDT) standard, which is the international zero point for the VCDT scale (Kaplan and Hulston 1966; Skrzypek and Dunn 2020b). In addition to $\delta(^{34}S)$, the stable oxygen

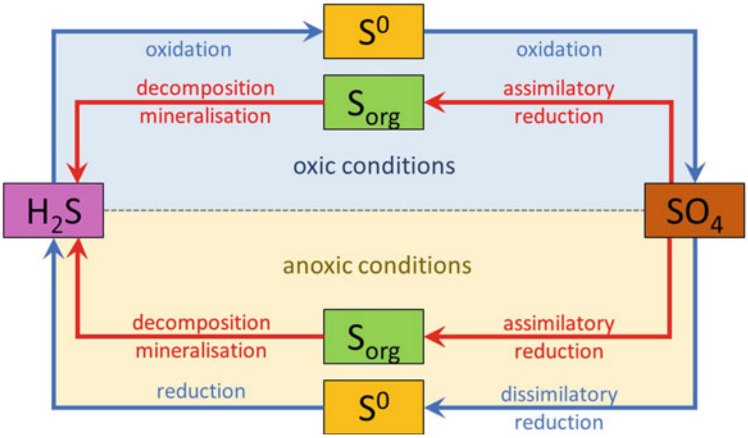

Fig. 6.1 Principles of sulfur biogeochemical cycling. Oxidation of hydrogen sulfide H_2S occurs in the presence of molecular O_2, leading to the production of sulfate (SO_4), with minor stable isotope fractionation. Sulfate (SO_4) can be utilized by plants via assimilatory reduction, again with minor stable isotope fractionation, and the resulting organic matter can be decomposed, leading to desulfurization. SO_4 is the compound preferred by plants uptake. Dissimilatory and assimilatory reduction can be conducted under anoxic conditions by a few groups of microorganisms using SO_4 as a biochemical substrate or during respiration. These biological processes are extremely ^{32}S selective and result in a very low $\delta(^{34}S)$ for H_2S and a very high $\delta(^{34}S)$ for residual SO_4

isotope composition of the oxygen bonded in sulfates $\delta(^{18}\mathrm{O})_{\mathrm{SO}_4}$ also provides valuable information about the sulfate origin. The $\delta(^{18}\mathrm{O})_{\mathrm{SO}_4}$ value is typically analyzed in relation to the stable oxygen isotope composition of the water in which the sulfate is dissolved and that of atmospheric oxygen. Oxygen has three stable isotopes with different abundances of $^{18}\mathrm{O} = 0.20\ \%$, $^{17}\mathrm{O} = 0.04\ \%$ and $^{16}\mathrm{O} = 99.76\ \%$ as mole fraction percentages (Coplen et al. 2002a). In the majority of studies, isotope delta values $\delta(^{18}\mathrm{O})$ reflecting the $^{18}\mathrm{O}/^{16}\mathrm{O}$ ratio are used and expressed in permille (‰) in relation to the international water standard representing the zero point on the Vienna Standard Mean Ocean Water (VSMOW) scale.

The range of $\delta(^{34}\mathrm{S})_{\mathrm{SO}_4}$ and $\delta(^{18}\mathrm{O})_{\mathrm{SO}_4}$ values observed in the environment arises from stable isotope fractionations occurring during chemical, physical, or biological processes. In general, the stable sulfur isotope fractionation is relatively small during sulfur oxidation processes (~0–2 ‰) and negligible during sulfate dissolution (Clark and Fritz 1997). By contrast, this fractionation is very large during bacterial sulfur reduction processes, at up to ~40 ‰, because bacteria preferentially take up the lighter $^{32}\mathrm{S}$ isotope, thereby leaving the residual sulfur enriched in the heavy $^{34}\mathrm{S}$ isotope (Habicht and Canfield 1997). $^{32}\mathrm{SO}_4{}^{2-}$ also reacts up to 1.07 faster than $^{34}\mathrm{SO}_4{}^{2-}$ (Weyer et al. 1979). During sulfur oxidation, oxygen forms a strong and stable chemical bond with sulfur and does not easily exchange with water under natural conditions (Fig. 6.1). Its $\delta(^{18}\mathrm{O})_{\mathrm{SO}_4}$ reflects the stable isotope composition of the oxygen source available during oxidation, which is usually atmospheric molecular oxygen or oxygen from water molecules. The isotope fractionation that occurs during the oxidation process depends on the source of oxygen and equals -11.4 ‰ between atmospheric oxygen and sulfate and $+4.1$ ‰ between water and sulfate (Taylor et al. 1984; Dogramaci et al. 2017).

6.2 Stable Isotope Composition of Sulfur Sources in Agro-ecosystems

Agro-ecosystems are usually studied on the catchment scale according to land use and water flow direction (Chap. 2). Therefore, the stable isotope and hydrochemical composition of pollution and natural solutes are monitored along waterways, in groundwater and pore water, and, if possible, at pollution sources or directly in contaminating compounds before their dispersal into the environment. The hydrochemical and stable isotope compositions of sulfate in water will therefore represent a mixture of various sources, both natural and anthropogenic, and the usual objective is to identify the fractional contributions from different sources and determine their locations. Hence, tracer studies very frequently use mass balance mixing models (Chap. 3) and sometimes include a stable isotope fractionation component (Lewicki et al. 2022). Mixing models can provide a fair assessment of the potential relative contributions from different sources if the hydrochemical and stable isotope signatures of each source are determined with reasonable accuracy. This is why the identification and characterization of potential sources are important before designing a specific sampling plan. The optimal method is to analyze the signature of each source at the studied location; however, the overview provided below can give benchmarks for the expected range of values.

The stable isotope studies using $\delta(^{34}\mathrm{S})_{\mathrm{SO_4}}$ and $\delta(^{18}\mathrm{O})_{\mathrm{SO_4}}$ as stable isotope hydrochemical tracers usually require consideration of the following potential sources of sulfur that contribute to waterways:

- ***Natural sources***
  - **Precipitation and sea spray**
  - **Rock weathering**
  - **Sediments and soils**
  - **Decomposing organic matter**
- ***Anthropogenic sources***
  - **Atmospheric pollution**
  - **Acid rock drainage due to mining or other excavations**
  - **Industrial pollution discharge**
  - **Agriculture fertilizers.**

6.2.1 Natural Sources

The sea spray contribution to the terrestrial sulfur budget is significant and occurs through the direct delivery of seawater droplets in coastal regions or by influencing the hydrochemical composition of precipitation. Sea spray delivers sulfate and dimethyl sulfate (DMS) at very high concentrations, but its delivery is restricted to coastal regions (Crawford et al. 2019; Lewis and Schwartz 2004). By contrast, precipitation that carries ocean signatures condenses across all land masses. It delivers large quantities of water, but the solutes are usually present at very low concentrations, with mean sulfate contents <3 mg/L and varying with distance from the coast and pollution sources; in pristine areas, the content is <0.3 mg/L (Vet et al. 2014). The generated loads of solutes are enormous on the landscape scale, but for sulfate surface budgets, these loads matter only in places with a long water retention time and a high evaporation rate (Skrzypek et al. 2013). In wet climates with high runoff, rainfall primarily causes solute dilution in the surface water, and its $\delta(^{34}\mathrm{S})_{\mathrm{SO_4}}$ and $\delta(^{18}\mathrm{O})_{\mathrm{SO_4}}$ are irrelevant due to the very low sulfate concentration (Vet et al. 2014). However, rainfall solute loads have a long-term impact on groundwater hydrochemistry in many regions, especially in arid and semiarid areas (Skrzypek et al. 2013, 2016), and in the absence of sulfur-bearing minerals in geological formations, rainfall may constitute a hydrochemical stable isotope baseline for sulfate in local pristine waters (Dogramaci and Skrzypek 2015).

The sulfate concentration in flood water depends on the evaporation and dissolution of salts deposited earlier on the ground; therefore, its concentration varies across a broad range and is not itself indicative of precipitation input. To make a meaningful estimation, other tracers can be used. For example, the ratio between sulfate and chloride (SO_4/Cl), expressed in milliequivalents (meq/L) in the range between 0.15 and 0.70, can be attributed directly to marine origin (Fig. 6.2). Higher ratios will suggest inputs other than rainfall sulfate, while lower ratios will imply sulfur removal by sulfate reduction or excessive halite dissolution. Oceans also have a very distinct compositions, with $\delta(^{34}S)_{SO_4}$ ~ 21.1 ‰ and $\delta(^{18}O)_{SO_4}$ ~ 8.6 ‰, and these are highly consistent around the world (Rees et al. 1978; Holser et al. 1979). The unique $\delta(^{34}S)_{SO_4}$ values progressively decrease from 21 ‰ at the coast to ~14 ‰ in inland locations, but they usually remain above these values for other sulfur sources (Chivas et al. 1991). This range of values can be used as a basic reference baseline for local studies (Fig. 6.2). In this example, samples with $\delta(^{34}S)_{SO_4}$ ~12 ‰ fall within the SO_4/Cl ratio typical for rainfall, suggesting precipitation as the dominant sulfate source for these waters (Fig. 6.2) (Dogramaci et al. 2017). By contrast, samples with a SO_4/Cl ratio significantly higher than 0.7 have low $\delta(^{34}S)_{SO_4}$ < 8 ‰, suggesting an input of sulfur with a low $\delta(^{34}S)_{SO_4}$.

Rock weathering occurs naturally and can represent an important source of sulfur in waterways (Calmels et al. 2007; Das et al. 2012; Szynkiewicz et al. 2015b). The common form of sulfur is sulfide (e.g., pyrite or chalcopyrite) in many types of rock, especially metamorphic and sedimentary rocks (Dogramaci et al. 2017). Elemental sulfur occurs less frequently in, for example, volcanic rocks and as sulfate

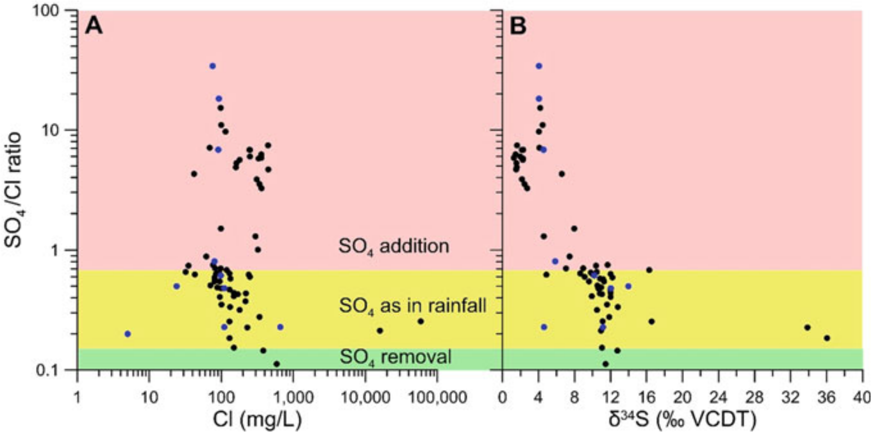

Fig. 6.2 Stable sulfur isotope composition and chloride concentration relative to the SO_4/Cl ratio (meq/L). The typical SO_4/Cl ratio for rainfall varies between 0.15 and 0.7. Samples with SO_4/Cl ratios >0.7 usually have additional S derived from sources other than precipitation. Samples with SO_4/Cl ratios <0.1 are subject to S removal due to sulfate precipitation [$\delta(^{34}S)_{SO_4}$ 10–15‰] or bacterial reduction [$\delta(^{34}S)_{SO_4}$ > 30‰]. Groundwater—black points, surface water—blue points. Data from Pilbara, Western Australia, figure modified after Dogramaci et al. 2017

in evaporative deposits or salt domes (gypsum). These different geological sources of sulfur frequently show distinct $\delta(^{34}\mathrm{S})_{\mathrm{SO_4}}$ and $\delta(^{18}\mathrm{O})_{\mathrm{SO_4}}$ values that can aid in the identification of the contribution to ground and surface waters. However, the natural variability has a broad range (Coplen et al. 2002b), so obtaining local values is important whenever practical (Szynkiewicz et al. 2015b).

Sulfides usually have very low $\delta(^{34}\mathrm{S})$, often below 0 ‰ (Krouse and Grinenko 1991; Clark and Fritz 1997), but values above zero have also been reported, such as −8.9 to 2.7 ‰ for the hydrothermal sulfides of the San Juan and Sangre de Cristo Mountains in the USA (Nordstrom et al. 2007; Campbell and Lueth 2008) or −1.9 to 4.4 ‰ for Pilbara in Western Australia (Dogramaci et al. 2017). In contrast to sulfides, the $\delta(^{34}\mathrm{S})_{\mathrm{SO_4}}$ sulfates of marine origin evaporites are usually near or above the seawater values and are typically >15 ‰ (Claypool et al. 1980). However, most evaporites from old rock formations can be partitioned from modern marine sulfates deposited with rainfall or sea spray based on $\delta(^{18}\mathrm{O})_{\mathrm{SO_4}}$. The evaporites $\delta(^{18}\mathrm{O})_{\mathrm{SO_4}}$ values are usually much higher than the theoretically expected value of 16 ‰ (Holser et al. 1979) due to diagenetic alterations, and their values are significantly above those of marine sulfates (8.6 ‰). The $\delta(^{34}\mathrm{S})_{\mathrm{SO_4}}$ values observed in sulfides may overlap with volcanic sulfur, which usually has values slightly above zero in both basalt rocks (Torssander 1988, 1989) and volcanic gases (De Moor et al. 2022).

The stable sulfur isotope composition of **soil and terrestrial sediments** primarily reflects geological sources and inputs from rain and sea spray (Mizota and Sasaki 1996; Stack and Rock 2011; Tanikawa et al. 2022) and consists of a mixture of minerals, organic sulfur or dissolved sulfates in porewater (Chalk et al. 2017). Under anoxic conditions (Fig. 6.1), if bacterial sulfate reduction occurs, the residual sulfate tends to have very high values, frequently >30‰ (Bottrell and Novak 1997; Oleinik et al. 2003; Schroth et al. 2007). Plant stable isotope composition usually reflects sulfur sources because the isotope fractionation occurring during sulfate uptake is usually negligible (Skrzypek et al. 2008; Chalk et al. 2017; Gałuszka et al. 2020). Therefore, plants do not provide unique signatures unless abundant organic matter decomposes under anoxic conditions, such as in wetlands or lake sediments, when the major driver of $\delta(^{34}\mathrm{S})$ is bacterial reduction causing very high $\delta(^{34}\mathrm{S})_{\mathrm{SO_4}}$ for residual sulfates (Trust and Fry 1992; Bottrell and Novak 1997; Skrzypek et al. 2010).

6.2.2 Anthropogenic Sources

6.2.2.1 Atmospheric Pollution

Atmospheric pollution in industrial areas frequently overwhelms the natural $\delta(^{34}\mathrm{S})_{\mathrm{SO_4}}$ signature of precipitation. Very high sulfate loads are still observed around the world, despite desulfurization technologies introduced in the 1980s–1990s to address the acid rain problem (Jędrysek et al. 2002a, b), and they can exceed 4 kg/ha for sulfur (e.g., in the Mideastern US), compared to ~0.1 kg/ha for sulfur in pristine areas (e.g., the Rocky Mountains) (https://enviroatlas.epa.gov). A similar or even

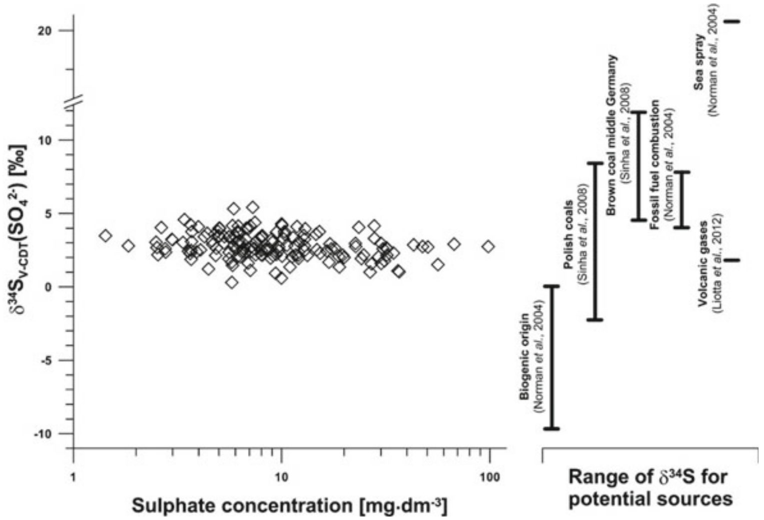

Fig. 6.3 Range of $\delta(^{34}S)_{SO_4}$ variation in precipitation collected in the city of Wroclaw (SW Poland) with respect to the values of potential sulfur sources. The observed lack of correlation between the SO_4 concentration and its $\delta(^{34}S)_{SO_4}$ suggests that pyrite oxidation during coal burning in a power plant is the main source of atmospheric sulfur, dominating sulfur budget in the atmosphere. Figure from Górka et al. (2017)

higher deposition of sulfur was observed in the industrial centers of Asia and Europe. The primary source of SO_2 emission in the atmosphere is coal combustion in power plants and smelters (Jędrysek et al. 2003). Sulfur in coal originates primarily from sulfides and therefore has a very low $\delta(^{34}S)$ (close to 0 ‰). During high-temperature combustion, whole sulfur is oxidized, and stable isotope fractionation for sulfur is low (Eldridge et al. 2016; Górka et al. 2017; Holt et al. 1982; Fig. 6.3). Sulfur is oxidized with atmospheric oxygen with a globally constant $\delta(^{18}O)$ value of 23.5–23.8 ‰ (Kroopnick and Craig 1972; Horibe et al. 1973), and with the addition of atmospheric moisture, this forms SO_4 characterized by a variable $\delta(^{18}O)$, but usually below 0 ‰ (Araguás-Araguás et al. 2000). As a consequence of these processes, the $\delta(^{34}S)_{SO_4}$ in precipitation in polluted areas is low and usually varies from 0 to 5 ‰, with seasonal variations and variations due to inputs from other sources of pollution (Jenkins and Bao 2006; Górka et al. 2017; Kim et al. 2018; Wu et al. 2021).

6.2.2.2 Acid Rock Drainage Due to Mining and Ground Disturbance

Sulfide oxidation occurs naturally through erosion processes of geological formations, which contribute to elevated concentrations of sulfates in the surface and groundwater (Cortecci et al. 2002; Brenot et al. 2007; Turchyn et al. 2013; Szynkiewicz et al. 2015b). The process occurring naturally on the ground surface primarily involves atmospheric oxygen, with only a small addition of oxygen from water molecules, at a proportion of 7:1 (Eq. 6.1). Therefore, during this reaction, only two moles of reduced hydrogen (H^+) are produced per mole of FeS_2 oxidized, and this contributes only moderately to water acidity. However, this process can escalate to a catastrophic scale when large amounts of pyrite are rapidly oxidized during large disturbances, such as those caused by mining, waste rock deposition, or construction work, that lead to acid rock drainage (ARD) (Dold 2017). This accelerated oxidation of a large amount of pyrite exposed to the surface during a disturbance results in a much lower pH than is observed with natural erosion processes occurring on the surface and on a small scale. Pyrite oxidation immobilizes iron, so the concentration of ferrous iron (Fe^{2+}) increases in the solution. Ferrous iron can subsequently be oxidized to ferric iron (Fe^{3+}) in the presence of oxygen at a low pH (Eq. 6.2), and this reaction can occur spontaneously or can be catalyzed by microorganisms. Ferric iron can also catalyze further pyrite oxidation under anoxic conditions using oxygen exclusively from water molecules and generating an eightfold higher acidity (Eq. 6.3) compared to oxidation involving atmospheric oxygen (i.e., 16 mol compared to 2 mol of H^+) (Eq. 6.1).

$$\mathrm{FeS_2(s) + 7/2O_2(g) + H_2O(l) = Fe^{2+}(aq) + 2SO_4^{2-}(aq) + 2H^+(aq)} \tag{6.1}$$

$$\mathrm{4Fe^{2+}(aq) + O_2(g) + 4H^+(aq) = 4Fe^{3+}(aq) + 2H_2O(l)} \tag{6.2}$$

$$\mathrm{FeS_2(s) + 14Fe^{3+}(aq) + 8H_2O(l) = 15Fe^{2+}(aq) + 2SO_4^{2-}(aq) + 16H^+(aq).} \tag{6.3}$$

These hydrochemical reactions will trigger elevated concentrations of sulfate and iron and low pH in waters impacted by ARD, which is accelerated by the presence of ferric iron. Low pH can be naturally neutralized in rock formations that contain large amounts of carbonates; therefore, acidity and sulfate concentrations alone cannot be used to verify the extent of ARD. For this reason, major ion hydrochemistry is frequently combined with stable isotope analyses. Pyrite in most geological formations has very low $\delta(^{34}S)$ usually close to 0 ‰ and the ARD process does not cause stable isotope fractionation (Taylor et al. 1984; Gałuszka et al. 2020). Therefore, the original $\delta(^{34}S)$ of pyrite is reflected in the initial $\delta(^{34}S)_{SO_4}$ value of the sulfate. However, some bacterially mediated reactions could be split into two phases, with some fractionation occurring during the initial stage (Brunner et al. 2008).

The $\delta(^{34}S)_{SO_4}$ value of produced sulfate usually does not overlap with other sources of natural sulfur in the environment or fertilizers. However, the $\delta(^{34}S)$ alone may not be indicative of different secondary sources of sulfates originating from sulfide oxidation. For instance, ARD usually cannot be partitioned from pyrite oxidation resulting from natural erosion or from air pollution carrying the signature of pyrite combusted with coal in power plants if the $\delta(^{34}S)$ values of the pyrite sources are similar. However, sulfate produced during sulfur oxidation in different environments will have a different $\delta(^{18}O)_{SO_4}$ that reflects the stable oxygen isotope composition in these environments. For example, sulfate oxidized with atmospheric oxygen will have values close to those of the oxygen in the atmosphere, which is constant around the globe (~23.5 ‰) (Kroopnick and Craig 1972) minus an isotope fractionation factor of -11.4 ‰ (Taylor et al. 1984). The stable oxygen isotope composition of surface water and groundwater is much more variable (usually -10 to $+10$ ‰) and depends on several factors, such as climate, distance from the sea, and evaporation losses (Gat 1997). Consequently, the $\delta(^{18}\mathrm{O})_{\mathrm{SO_4}}$ will reflect the signature of the water available at the time of oxidation plus a fractionation factor of $+4.1$ ‰ (Taylor et al. 1984).

Oxygen and sulfur form strong chemical bonds in sulfate; therefore, both $\delta(^{34}\mathrm{S})_{\mathrm{SO_4}}$ and $\delta(^{18}\mathrm{O})_{\mathrm{SO_4}}$ are well preserved in solution unless sulfate is subject to biological processes and significant reduction, especially in anoxic conditions (Fritz et al. 1989, Fig. 6.1). Based on the stable isotope signatures of contrasting sources of oxygen contributing to sulfide oxidation, the contributions from the two leading processes to ARD can be partitioned (Eqs. 6.1–6.3).

6.2.2.3 Agriculture Fertilizers

The stable sulfur and oxygen isotope compositions of fertilizers vary in relatively large ranges according to their chemical compositions, manufacturing processes used for synthetic fertilizers and mineralogy of natural fertilizers (Mizota and Sasaki 1996; Coplen et al. 2002c; Vitòria et al. 2004; Chalk et al. 2017). This variability in $\delta(^{34}\mathrm{S})_{\mathrm{SO_4}}$ and $\delta(^{18}\mathrm{O})_{\mathrm{SO_4}}$ underscores the importance of understanding what types of fertilizers are used locally and, if possible, analyzing their stable isotope compositions. However, both $\delta(^{34}\mathrm{S})_{\mathrm{SO_4}}$ and $\delta(^{18}\mathrm{O})_{\mathrm{SO_4}}$ fertilizer signatures have been successfully used in several studies to partition agriculture sources from other sulfur sources by applying mixing models and estimating inputs from agrocontaminants (Mizota and Sasaki 1996; Hosono et al. 2007; Nakano et al. 2008; Szynkiewicz et al. 2015a; Urresti-Estala et al. 2015).

The dissolution and leaching of fertilizers to the surface and groundwater do not cause isotope fractionation, so the generated dissolved sulfate reflects the initial signature of the fertilizers (Chalk et al. 2017). However, the stable isotope composition of leachates, and especially $\delta(^{18}\mathrm{O})_{\mathrm{SO_4}}$, can be further modified by bacterial processes. Hence, besides the initial stable isotope composition of fertilizers, processes contributing to the stable isotope fractionation of sulfates in soil and aquatic environments should also be considered. Stable isotope fractionation caused by microbiological processes adds uncertainty to the calculations, but the fertilizers usually still have a distinct composition that partitions them from other sources of sulfur in agrosystems if both $\delta(^{34}\mathrm{S})_{\mathrm{SO_4}}$ and $\delta(^{18}\mathrm{O})_{\mathrm{SO_4}}$ were analyzed at the same time (Fig. 6.4). The $\delta(^{34}\mathrm{S})_{\mathrm{SO_4}}$ values are usually reported in the literature (Mizota and Sasaki 1996; Chalk et al. 2017), but relatively limited $\delta(^{18}\mathrm{O})_{\mathrm{SO_4}}$ data are available (Vitòria et al. 2004). The median $\delta(^{34}\mathrm{S})_{\mathrm{SO_4}}$ value reported by Vitòria et al. (2004) in the data compilation from a few countries, but primarily from Spain, was 5.7 ‰. The majority of fertilizers (25 of the 27 analyzed) from Spain had $\delta(^{34}\mathrm{S})_{\mathrm{SO_4}}$ between $-$

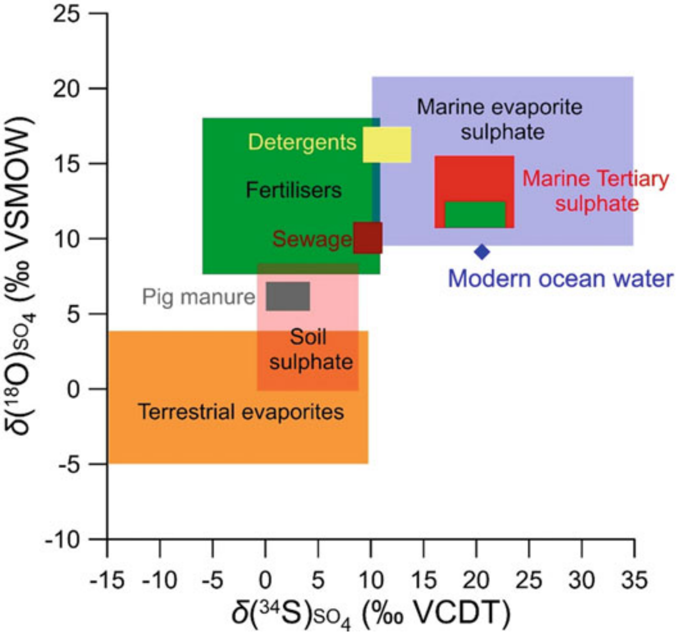

Fig. 6.4 Range of stable sulfur and oxygen isotope compositions of fertilizers based on the compilation made by Vitòria et al. (2004), and Urresti-Estala et al. (2015), in reference to other typical sources of sulfate found in agrosystems. Note that fertilizers are represented by two separate green polygons according to origin

1.8 and 11.7 ‰ and $\delta(^{18}O)_{SO_4}$ between 7.7 and 16.5 ‰, thereby distinguishing these fertilizers from many other sulfur sources.

The type of applied fertilizer depends on the type of agricultural production, soil, and climate. Thus, the relatively broad range of $\delta(^{34}S)_{SO_4}$ and $\delta(^{18}O)_{SO_4}$ presented in Fig. 6.4 could be much more restricted for local studies on the catchment scale. Further, the subdivision of catchments according to the water flow direction and land use (Chap. 2) can provide even narrower value ranges for local use in mixing models developed for a particular location (Chap. 3 and Fig. 6.5).

6.2.2.4 Other Industrial Pollution

Some other industrial pollutants impact the environment and influence $\delta(^{34}S)_{SO_4}$; however, these impacts occur less frequently and on more restricted scales. Some of these pollutants (e.g., hydrocarbons), even those characterized by low sulfur concentrations, can create anoxic conditions that accelerate microbiological sulfur reduction, thereby leading to substantial stable isotope fractionation. Specific chemical compounds in industrial pollution can also be used to determine dispersal pathways (Santucci et al. 2018; Schroth et al. 2001; Torssander et al. 2006).

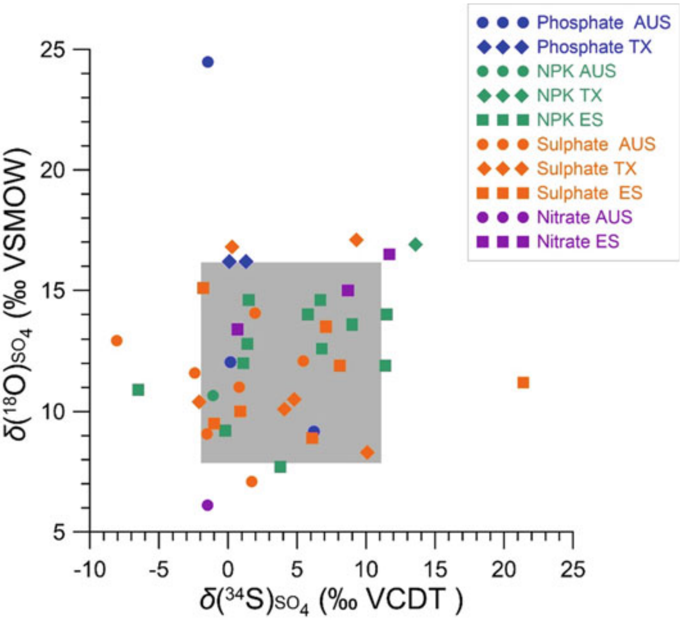

Fig. 6.5 Stable sulfur and oxygen isotope compositions of 43 fertilizer samples reported for Texas (Szynkiewicz et al. 2015a), Spain (Vitòria et al. 2004) and Western Australia (Data from Department of Water and Environmental Regulation and Department of Primary Industries and Regional Development of Western Australia). The gray area represents 80 % of the sample number with respect to the value range. Symbols indicate countries, and colors indicate chemical compositions

6.3 Disentangling Acid Rock Drainage from Agrocontaminants Using $\delta(^{2}\mathrm{H})_{\mathrm{H_2O}}$, $\delta(^{18}\mathrm{O})_{\mathrm{H_2O}}$, $\delta(^{34}\mathrm{S})_{\mathrm{SO_4}}$ and $\delta(^{18}\mathrm{O})_{\mathrm{SO_4}}$

The stable isotope analyses of sulfate usually are accompanied by analyses of stable hydrogen and oxygen isotope composition of water. The obtained $\delta(^{2}\mathrm{H})_{\mathrm{H_2O}}$, $\delta(^{18}\mathrm{O})_{\mathrm{H_2O}}$ results can support interpretation of $\delta(^{34}\mathrm{S})_{\mathrm{SO_4}}$ and $\delta(^{18}\mathrm{O})_{\mathrm{SO_4}}$ values and better partition the fate of water molecules from the fate of solutes. This is particularly important for interpretation of $\delta(^{18}\mathrm{O})_{\mathrm{SO_4}}$ for which understanding of $\delta(^{18}\mathrm{O})_{\mathrm{H_2O}}$ is required and variability in water stable isotope composition of major water sources.

6.3.1 *Principles of $\delta^{2}H(H_2O)$ and $\delta^{18}O(H_2O)$ Interpretation*

The stable hydrogen and oxygen isotope composition of water, $\delta(^{2}\mathrm{H})_{\mathrm{H_2O}}$ and $\delta(^{18}\mathrm{O})_{\mathrm{H_2O}}$, can inform us about the water sources contributing to a water body and distinguish inputs originating from four usually considered sources: (1) precipitation, (2) groundwater, (3) surface runoff, and (4) ocean water. Each of these sources can have very distinct $\delta(^{2}\mathrm{H})_{\mathrm{H_2O}}$ and $\delta(^{18}\mathrm{O})_{\mathrm{H_2O}}$, but their values vary spatially and temporarily with respect to the major drivers governing precipitation, groundwater recharge conditions, and evaporation, reflecting retention time on the ground surface (Gat 1997). All these values should be considered based on local conditions and the Local Meteoric Water Line (LMWL) and Local Evaporation Line (LEL), as defined by $\delta(^{2}\mathrm{H})$–$\delta(^{18}\mathrm{O})$ relationship, as well as the precipitation pattern prior to sampling (Fig. 6.6; Craig and Gordon 1965; Rozanski et al. 1993).

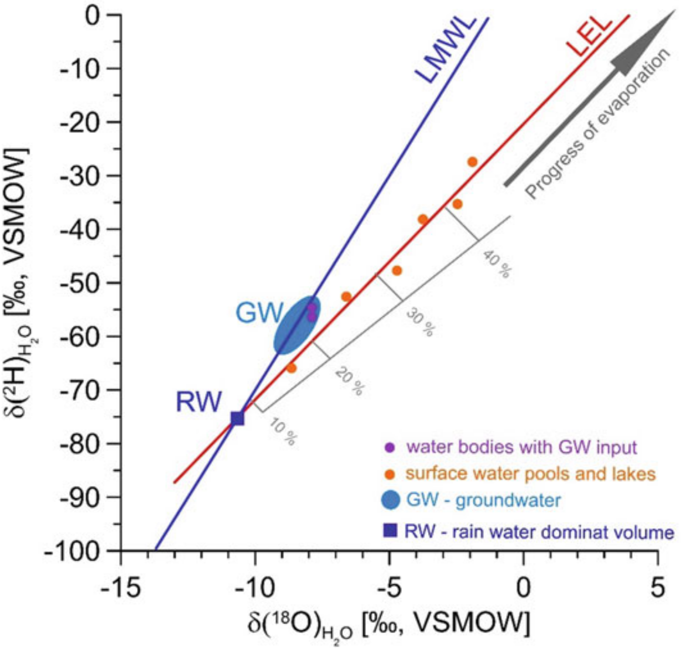

Fig. 6.6 Conceptual plot of the stable hydrogen and oxygen isotope composition of major water budget components considered for the evaluation of water source mixing and the progress of evaporation. The presented numerical range areas are those observed for the Pilbara region of Western Australia; these ranges will vary according to the local conditions. The slope of the Local Meteoric Water Line (LMWL) usually varies by ~8 to ~7 and the Local Evaporation Line (LEL) slope varies by ~5 to ~4. The dominant precipitation resulting from large-volume events or precipitation during cold periods tends to have negative values (RW). Groundwater (GW) usually reflects the values of the dominant precipitation, lies close to LMWL and has negative values. However, it is higher than RW and reflects the evaporative loss occurring between precipitation and infiltration into the groundwater aquifer. The stable isotope compositions of lakes and other water bodies are distributed along the LEL, and their position reflects the progress of evaporation, with higher delta values for more evaporated waters. Water bodies with significant additions of GW tend to lie between LEL and LMWL and close to GW. The crossing point between LEL and LMWL indicates the initial value for water prior to evaporation and lies close to RW

Precipitation varies seasonally according to temperature, humidity, source of moisture, rainout effect, elevation, distance from the coast, and other parameters (Dansgaard 1964; Rozanski et al. 1992, 1993). According to the International Atomic Energy Agency (IAEA) guidelines, the most appropriate method is to obtain LMWL locally from the monthly mean precipitation collected over a period of a minimum of three years (Gröning et al. 2012; Hughes and Crawford 2012; IAEA 1992). However, if local data are not available, several locations around the world have at least some precipitation data accessible from the Global Network of Isotopes in Precipitation (GNIP) database (Schotterer et al. 1996, https://www.iaea.org/services/networks/gnip) or national datasets (for example, in Australia: Hollins et al. 2018, in Canada: Peng et al. 2004, in Nigeria: Ohwoghere-Asuma et al. 2021, in the USA: Putman and Bowen 2019; Tian and Wang 2019 and in Vietnam: Le Duy et al. 2018). For regions lacking local data, approximate values can be obtained using an interpolation algorithm, as proposed by Bowen et al. (Bowen 2019; Bowen and Revenaugh 2003; https://waterisotopes.org).

Groundwater primarily reflects the stable isotope composition of the dominant precipitation contributing to recharge, such as large-volume events during the wet season (Skrzypek et al. 2019), snowmelt (Jasechko et al. 2017b) or past periods of abundant precipitation forming paleogroundwater (Jasechko et al. 2017a). These large-volume events or wet periods tend to have negative signatures. Similarly, at higher latitudes, the isotope composition is biased toward precipitation during the cold season and therefore also tends to have more negative values than during other seasons (Marchetti and Marchetti 2019). Therefore, groundwater frequently has low $\delta(^{2}\mathrm{H})_{\mathrm{H_2O}}$ and $\delta(^{18}\mathrm{O})_{\mathrm{H_2O}}$ compared to surface water, and these values are consistent on a regional or basin scale (Dogramaci et al. 2012a).

The surface water stable isotope composition in lakes and rivers is governed by evaporation and primarily reflects retention time (Fellman et al. 2011). The progress of evaporation reflects climate conditions, and retention time, since precipitation or water influx can be estimated by applying the Craig-Gordon model (Craig and Gordon 1965). Sampling inflow and outflow from a lake and comparing $\delta(^{2}\mathrm{H})_{\mathrm{H_2O}}$ and $\delta(^{18}\mathrm{O})_{\mathrm{H_2O}}$ allows for the estimation of evaporation over the inflow ratio. Similarly, repetitive sampling of a lake, pond, or waterhole as the water level decreases due to evaporation allows estimation of the water fraction loss (Fellman et al. 2011; Nitzsche et al. 2017; Paulsson and Widerlund 2020). Evaluation of evaporative losses helps to define the water budget for surface water bodies (Yapiyev et al. 2020) and to partition the fate of water molecules from the fate of solutes. The calculation procedure employing the C-G model has been simplified and standardized by the introduction of Hydrocalculator software (Skrzypek et al. 2015).

The ocean water $\delta(^{2}\mathrm{H})_{\mathrm{H_2O}}$ and $\delta(^{18}\mathrm{O})_{\mathrm{H_2O}}$ values are usually constant over a long time at a given location, and they reflect latitude. The mean ocean water $\delta(^{2}\mathrm{H})_{\mathrm{H_2O}}$ and $\delta(^{18}\mathrm{O})_{\mathrm{H_2O}}$ values are equal to 0‰ and define the zero point for the VSMOW2-SLAP2 international stable hydrogen and oxygen isotope scale (Coplen 1995). However, the surface water may vary locally with respect to evaporation, freshwater input, and ice melt, with values usually between −1 and 2‰ for $\delta(^{18}\mathrm{O})_{\mathrm{H_2O}}$, and between −3 and 10 ‰ for $\delta(^{2}\mathrm{H})_{\mathrm{H_2O}}$ (Schmidt 1999; Benetti et al. 2017). The ocean stable isotope composition could be used to assess mixing in estuaries (Price et al. 2012) or groundwater intrusion (Heilweil et al. 2009; Carreira et al. 2014) and to assess solute inputs in coastal regions.

6.3.2 Combined Approach to Partition Sulfate Sources Using Hydrochemical Composition, $\delta(^{18}O)_{H_2O}$ and $\delta(^{18}O)_{SO_4}$

Stable isotope analyses of $\delta(^{2}\mathrm{H})_{\mathrm{H_2O}}$ and $\delta(^{18}\mathrm{O})_{\mathrm{H_2O}}$ have become routinely used tools to supplement major ion analyses. They can be coupled with analyses of changes in salinity, especially targeting conservative ions such as Cl to distinguish evapoconcentration from inputs other than precipitation (e.g., recycled accumulated salt or salt originating from geological formation or ions contributing from pollution) (Skrzypek et al. 2013). In pollution studies, $\delta(^{18}\mathrm{O})_{\mathrm{H_2O}}$ can also be used to track the process of sulfate formation because $\delta(^{18}\mathrm{O})_{\mathrm{SO_4}}$ primarily reflects the stable isotope composition of water and, to some extent, atmospheric oxygen, which is characterized by very constant values of ~23.5 ‰ (Kroopnick and Craig 1972). Following Eqs. 6.1–6.3, the contribution from these two oxygen sources to sulfate formation can be used to assess the dominant reaction for sulfate formation and the risk of ARD.

The potential of acid rock drainage is usually investigated in six steps and can be further used to partition other sources of sulfates in waterways:

1. The $\mathrm{SO_4}$/Cl ratio (Fig. 6.2a) indicates the possibility of sulfate addition above those attributed to rainfall input. If the $\mathrm{SO_4}$/Cl is higher than 0.7, then part of the sulfate could originate from a source that does not contribute to other major conservative ion concentrations (e.g., conservative Cl). This is informative regarding the excessive input above that is observed in precipitation, but it does not specifically identify the source. However, other hydrochemical models can provide additional information about potential sources (Dogramaci et al. 2012b).
2. The decrease or increase in $\delta(^{34}\mathrm{S})_{\mathrm{SO_4}}$ correlated with an increased concentration of $\mathrm{SO_4}$ could also be attributed to the signature of the dominant sulfur input source (Fig. 6.7). Sulfides usually tend to have low or negative $\delta(^{34}\mathrm{S})_{\mathrm{SO_4}}$. Therefore, a decrease in values is frequently associated with leaching from pyrite oxidation (e.g., García-Lorenzo et al. 2014; Dogramaci et al. 2017; Chudy et al. 2021). The best option is to confirm the local signatures of pyrite by directly analyzing minerals from the rock matrix in the study area.
3. In many cases, pyrite has a distinct signature that is lower than that of many other sources of sulfur. Therefore, $\delta(^{34}\mathrm{S})_{\mathrm{SO_4}}$ alone could be sufficient to partition pyrite sulfur input from many other sources (García-Lorenzo et al. 2014; Chalk et al. 2017; Chudy et al. 2021). However, the values can overlap with fertilizers, which also tend to have low $\delta(^{34}\mathrm{S})_{\mathrm{SO_4}}$ in some cases (Fig. 6.4). Fertilizer contributions can be separated from pyrite contributions using $\delta(^{18}\mathrm{O})_{\mathrm{SO_4}}$, as the $\delta(^{18}\mathrm{O})_{\mathrm{SO_4}}$

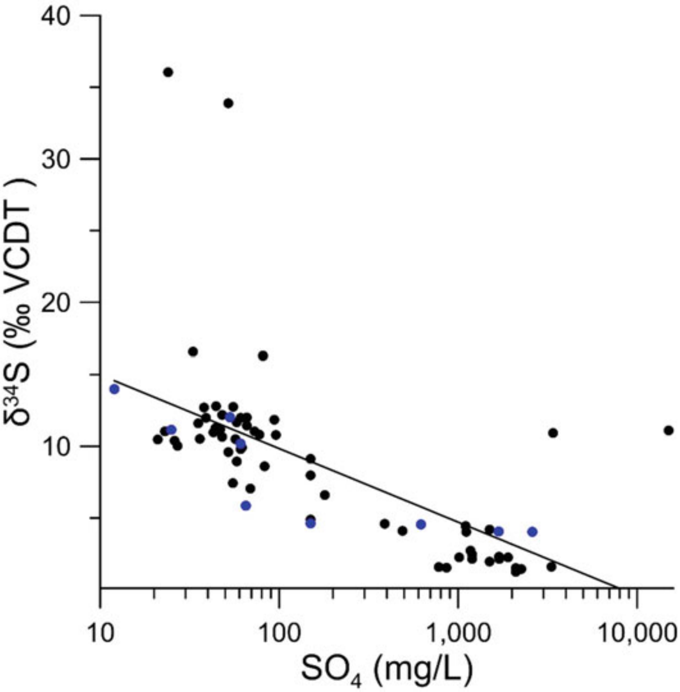

Fig. 6.7 Increases in sulfide concentration correlate with decreases in $\delta(^{34}S)_{SO_4}$ in a case study from Pilbara, Western Australia. The pH at most locations was neutral because of buffering by carbonates. Acid rock drainage had to be confirmed using a combination of various hydrochemical and stable isotope tracers (blue points—surface water, black points—groundwater). Figure from Dogramaci et al. (2017)

originating from pyrite oxidation is usually low (<10 ‰) and therefore different from most fertilizers (>10 ‰) (Fig. 6.4).

4. The extent of ARD can be further confirmed using $\delta(^{18}O)_{SO_4}$ linked with $\delta(^{18}O)_{H_2O}$ of local ground or surface water in which sulfates are dissolved (Toran 1987; Taylor et al. 1984). The model proposed by Taylor et al. (1984) allows assessment of what portions of oxygen in the generated sulfate originate from the atmosphere and from water with respect to Eqs. 6.1–6.3 (Fig. 6.8). However, $\delta(^{18}O)_{SO_4}$ can be prone to microbial alterations by sulfuric bacteria, so $\delta(^{18}O)$ will be replaced by the value of water available for the metabolic processes of bacteria in those cases; therefore, it should be treated with caution particularly when sulfate concentrations in water are low (Brunner et al. 2008). This method is still useful for assessment of ARD extent if uncertainty associated with potential bacterial reduction is kept in mind.
5. The results of the ARD sulfate contribution could be used as input values for mixing models to calculate the proportional contributions from all determined

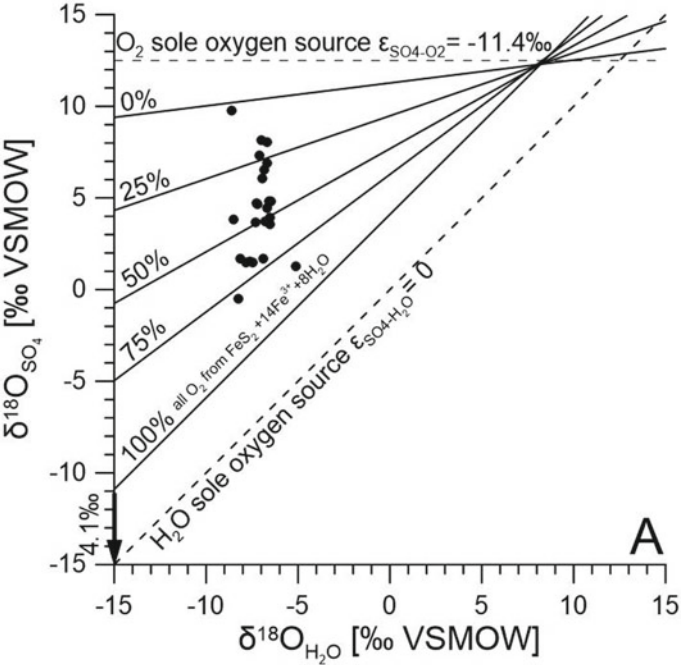

Fig. 6.8 Estimation of the acid rock drainage (ARD) contribution to groundwater sulfates based on the stable oxygen isotope compositions of water and sulfate. Low $\delta(^{18}\mathrm{O})_{\mathrm{SO_4}}$ and $\delta(^{18}\mathrm{O})_{\mathrm{H_2O}}$ tend to suggest higher inputs from ARD (points between 50 and 100 %). Figure after Dogramaci et al. (2017)

sulfur sources, as discussed in Chap. 3. To account for uncertainty in the calculation, the average value for each considered source should be used, as well as a range of values reflecting the natural variability in each source $\delta(^{34}\mathrm{S})_{\mathrm{SO_4}}$ and $\delta(^{18}\mathrm{O})_{\mathrm{SO_4}}$.

The potential of ARD using the above principles can be qualitatively evaluated using a few categories with respect to simple hydrochemical parameters and sulfate and water stable isotope compositions, using the classification proposed by Dogramaci et al. (2017).

The principles summarized above have been successfully used in several variants of studies that endeavored to partition ARD sulfur sources from other inputs, usually to complement hydrochemical analyses of major ions and nutrients. The application of multiple tracers at the same time allows a more robust determination of contributions from various sources and a clearer estimation of calculation uncertainty. These outcomes help to evaluate what types of factors and human activities impact water quality. Szynkiewicz et al. (2015a, b) comprehensively assessed several sulfur sources in the Rio Grande catchment by partitioning sulfide inputs from agriculture fertilizer leachates, and they reported that the SO_4 fluxes in Rio Grande at El Paso, at

200–450 metric tons/day, were originating from agrocontaminates primarily associated with irrigation periods linked with intensive fertilizer application. By contrast, this flux was only 30–80 metric tons/day during the postharvest winter season, when the majority of sulfate in the river was delivered by natural brines seeping into the catchment. At the same time, in the upper part of the Rio Grande catchment, sulfides contributed 83–94 % of the total sulfate budget (Szynkiewicz et al. 2015a, b). Toran (1987) was able to identify an area of sulfate contaminatioûn in groundwater from a carbonate-hosted mine on farmland in Wisconsin. Using a mixing model, Toran (1987) also applied hydrochemical modeling using the USGS computer program PHREEQE and determined the contribution from acid rock drainage despite the neutral pH due to buffering by carbonate rocks. Newman et al. confirmed underground seepage from an acidic pit lake to a perennial stream system. The acid rock drainage water input elevated the sulfate concentration and lowered the pH, which contributed to the mobility of potentially toxic metals (Al, Cd and Zn). The use of stable isotope methods allowed the evaluation of the extent of mining impacts in the catchments downstream from the mine and the partitioning of mining sources from other sulfur sources (Newman et al. 2020) and separate mining inputs from other S-sources including agriculture. A similar approach was adopted by Chudy et al. (2021) who assessed the impact of an abandoned iron ore mine on water quality. The extent of the mining influence and separation from the geological background could be further evaluated using bioindicator methods and plant tissue analyses (e.g., Migaszewski and Gałuszka 2019; Gałuszka et al. 2020).

6.4 Sample Preparation Techniques and Measurements

The sample of sulfate used for $\delta(^{34}\mathrm{S})_{\mathrm{SO_4}}$ and $\delta(^{18}\mathrm{O})_{\mathrm{SO_4}}$ analyses is usually precipitated from water as barium sulfate ($BaSO_4$). Several variants of the procedure have been described in the literature, but all are based on the steps originally designed for the classical method for the analysis of sulfate concentrations (e.g., Carmody et al. 1998; Skrzypek 2018):

1. Usually, between 0.5 and 4 L of water sample is collected (for SO_4^{2-} concentration, this range is for 50 and 5 mg/L of sulfate, respectively) to clean high-density polyethylene (HDPE) bottles. Bottles should be filled full, without headspace, and well-sealed. These water volumes are sufficient to precipitate ~50 mg of $BaSO_4$. For a single stable isotope analysis, usually up to 1 mg of $BaSO_4$ is required, but handling such small aliquots is impractical during washing and transferring. Water samples should be filtered through 0.45 μm filters in the field or as soon as delivered to the laboratory. Sulfate samples are prone to stable isotope fractionation caused by bacterial reactions; therefore, they should be stored chilled on ice in a portable ice chest box in the field and later in a fridge at ~+ 5 °C.

2. Before $BaSO_4$ is precipitated, the pH of the filtered sample should be adjusted by adding a few milliliters of HCl to lower the pH to 2–3. The volume of acid required depends on the HCl concentration used and water alkalinity, but typically ~3–5 mL of 5 N HCl is sufficient.
3. To accelerate crystal formation, warming the sample to ~40 °C is recommended. Precipitation of 50 mg of $BaSO_4$ (as per the volumes and concentrations listed in point 1) requires the addition of ~1.2 mL of 10 % $BaCl_2$; this volume contains approximately three times more barium than required by the stochiometric reaction to ensure fast and complete precipitation and also to take into consideration the potential uncertainty in the prediction of sulfate concentration. After vigorous stirring with a glass rod, the solution needs to be left for several hours to allow barium sulfate formation and deposition at the bottom of the beaker.
4. The following day, white crystals should cover the bottom of the beaker and the supernatant should be transparent. The completeness of sulfate precipitation can be tested by adding a small amount of $BaCl_2$ solution to see if further precipitation occurs, appearing as foggy tracers in the supernatant. In the next step, the supernatant needs to be removed and the white $BaSO_4$ crystals washed with deionized water at least three times. The supernatant removal procedure can be performed using a simple siphon tube and then the beaker is refilled with deionized water. Again, the sample needs to be left overnight for crystal sedimentation. This process needs to be repeated three times. Alternatively, to accelerate the washing process, the sample can be transferred to centrifuge tubes, spun and the supernatant removed and replaced with deionized water. Using a centrifuge at 5000 rpm accelerates the separation of crystals and the supernatant and eliminates the overnight wait time. The washing with deionized water and centrifugation procedure should be repeated three times to remove traces of HCl and any unreacted excess of $BaCl_2$.
5. After washing, the collected $BaSO_4$ sample can be transferred to a small evaporative dish and dried at 110 °C in a laboratory oven. After drying and cooling, the samples can be stored in small glass or plastic vials for several years. Pure $BaSO_4$ should be white (or light gray) powder, like fine kitchen salt.
6. $BaCl_2$ is toxic, particularly for aquatic organisms. Therefore, all wastewater from washing needs to be neutralized by adding H_2SO_4 to precipitate the residual barium remaining in solution, and then the pH can be adjusted to neutral using sodium carbonate (e.g., soda ash).

The procedure presented above does not account for potential interferences that can be caused by compounds other than sulfate sulfur and oxygen-bearing compounds present in water samples (Skrzypek 2018). Therefore, modification of this procedure may be required to address the following potential problems:

1. Samples that contain large concentrations of dissolved organic carbon (DOC) may be contaminated by coprecipitated organic fractions, as indicated by frequent changes in sample color from white to dark gray or brown. To address this issue, prefiltration through a 0.21 μm filter can be considered. Contaminated samples can also be further processed by adding 10 % hydrogen peroxide (H_2O_2) to an

evaporation dish with dry $BaSO_4$ to oxidize organic matter. The small addition of organic matter usually has a minor influence on $\delta(^{34}S)_{SO_4}$ but could significantly change $\delta(^{18}O)_{SO_4}$.

2. If the H_2S concentration in water samples is >0.5 mg/dm^3 (characterized by a distinctive rotten-egg odor), H_2S needs to be removed by precipitation and filtration to separate it from $SO_4{}^{2-}$. The water sample must first be transferred to a glass beaker (500 or 1000 mL depending on the sample volume) and 5% zinc acetate solution added in a 1:10 ratio (e.g., 50 mL zinc acetate solution to 500 mL of water sample volume). After mixing, at least 1 h should be allowed for precipitation and sedimentation (Carmody et al. 1998).
3. Analyses of $\delta(^{18}O)_{SO_4}$ in water samples that contain high concentrations of nitrates can be impacted by coprecipitation of nitrates that inoculate crystals of $BaSO_4$. Nitrates will yield oxygen during the analysis, and they will be added to the oxygen yield from sulfates. In coprecipitated NO_3/SO_4 mixtures where molar ratios are above 2, the oxygen bonded in nitrates may contribute up to 7 % of the total yields and cause changes in $\delta(^{18}O)_{SO_4}$ of up to 5 ‰ (Michalski et al. 2008). Nitrates can be removed from water before precipitation of barium sulfate using the anion-exchange resin method (Le Gendre et al. 2017) or after precipitation using a chelating solution to redissolve and then reprecipitate barium sulfate (Bao 2006).

The stable sulfur and oxygen isotope compositions of sulfate in barium sulfate samples are routinely analyzed using IRMS in traditional offline systems or in continuous flow mode (Sharp 2017). In the currently more popular continuous flow systems, $BaSO_4$ for $\delta(^{34}S)_{SO_4}$ analyses is decomposed at a high temperature (1000 °C) in elemental analyzers adapted for sulfur. After purification and water removal, SO_2 gas in the helium stream is introduced to the ion source of a mass spectrometer. Analyzing SO_2 compared to the SF_6 gas traditionally used in offline systems is challenging due to isobaric interferences caused by the variable stable oxygen isotope composition. This arises from the physical properties of SO_2, as different combinations of sulfur and oxygen isotopes can result in the same masses of SO_2 molecule (Sharp 2017). This issue can be partially overcome using various correction factors and utilizing IRMS systems with three collectors that analyze simpler molecules (e.g., fragmented ions, such as SO^+ with possible masses 48, 49 and 50). This analytical approach simplifies the required isobaric corrections and reduces analytical uncertainty. The analyzed samples are normalized to the VCDT scale for presentation as isotope delta values (Skrzypek et al. 2022) using at least two, but preferably three, well-calibrated laboratory or international standards, such as NBS127, IAEA-S-1, IAEA-S-2, and IAEA-S-3 (Skrzypek 2013). The usual combined analytical uncertainty of $\delta(^{34}S)_{SO_4}$ analysis varies between 0.3 and 0.4 ‰. The stable oxygen isotope composition of sulfates, $\delta(^{18}O)_{SO_4}$, can also be analyzed using continuous flow IRMS systems that differ from those used for $\delta(^{34}S)_{SO_4}$. For $\delta(^{18}O)_{SO_4}$ analysis, high-temperature pyrolysis (HTP) at 1450 °C is required, utilizing a thermal conversion elementary analyzer (TC/EA). $BaSO_4$ is reduced in the presence of hot graphite and glassy carbon, and carbon monoxide (CO) is produced. CO is separated from other gases using a gas chromatography column and introduced to the ion source of the IRMS. After multipoint normalization (Skrzypek 2013), usually based on international standards (e.g., IAEA-SO5, IAEA-S06 and NBS127), the results are reported on the VSMOW stable isotope scale with a combined analytical uncertainty usually 0.30–0.40 ‰, one sigma (Skrzypek and Sadler 2011).

6.5 Limitations and Challenges

Stable isotope delta results are reported with respect to entirely artifact-based scales that occasionally require updates (e.g., VSMOW2-SLAP2 or VPDB). To ensure the longevity of published data, a comprehensive description must be provided of (1) the analytical procedure; (2) traceability; (3) data processing; and (4) the uncertainty evaluation. For the minimum requirements for publishing stable isotope results, please refer to the guidelines prepared by the International Atomic Energy Agency and the International Union of Pure and Applied Chemistry (Skrzypek et al. 2022). If recalculations are required between different expressions of the stable isotope composition (e.g., isotope ratio, isotope fraction, isotope delta), the differences between the absolute values of the zero points for stable isotope scales adopted in the manufacturing software setup need to be considered and the calculations adjusted accordingly (Skrzypek and Dunn 2020a, b).

In catchments contaminated with pollutants originating from several sources, the major challenge is clarifying the ambiguous stable isotope signatures of overlapping values. To a large extent, this issue can be addressed using denser sampling to determine pollution budgets in individual subcatchments and by applying more advanced probabilistic models rather than simple mass balance arithmetic calculations (Lewicki et al. 2022).

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G. Skrzypek has 24 years of experience in the field of stable isotope geochemistry, hydrochemistry, and ecology since his PhD (1999) from The University of Wrocław (UWr). Grzegorz is currently working as Associate Professor and Coordinator of M.Sc Biology and Ecology specializations at The University of Western Australia (UWA). Grzegorz has published many papers on applications of stable isotope techniques in hydrological, hydrochemical, pollution, ecological, and paleoclimate studies. He uses stable H, C, N, O, S, and Sr isotopes to trace water budget, nutrient fluxes in natural environments, and dispersal of pollution in environments impacted by mining and agriculture. He held a prestigious Australian Research Council Future Fellowship at UWA (2012–2015) and Foundation for Polish Science Scholarship at UWr (1999–2000). Currently, he is serving as the deputy director of the West Australian Biogeochemistry Centre at UWA (wabc.uwa.edu.au). Grzegorz has been a chief investigator on a number of research projects in Europe and Australia and an expert on IAEA Technical Cooperation Projects and IAEA Technical Meetings.

Chapter 7
Quantifying Sediment and Associated Pollutants Sources in Agricultural Catchments Using Isotopic Techniques

W. H. Blake, A. Taylor, E. Muñoz-Arcos, L. Ovando-Fuentealba, C. Bravo-Linares, and G. E. Millward

Abstract This chapter provides an overview of nuclear tools used in sediment source apportionment studies, summarising essential information about the use of fallout radionuclides, elemental geochemistry, and compound-specific stable isotopes. Exemplar studies are described to show how the method can inform decision making at farm and basin scales to control diffuse water pollution by agriculture.

7.1 Overview of Sediment and Associated Pollutants in Agricultural Catchments

In the context of agricultural pollution, sediment derived from soil erosion is widely regarded as a major pollutant both in its own right and in its role as a vector for particle-reactive agrochemicals. The widespread and costly environmental impacts of sediment transfer from agricultural land into downstream aquatic ecosystems are recognised by river basin managers and policy makers worldwide (Evans 2010), yet

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the complexity of sediment production and delivery processes renders it a complex challenge to mitigate. Soil erosion by water poses a serious threat to water and nutrient retention, biodiversity, and plant primary productivity on agricultural land (Pimentel 2006). Downstream, sediment, and nutrient export to rivers and lakes contributes to eutrophication and contamination of water resources by algae and associated natural toxins, affecting the functioning of wetland and lake ecosystems. Furthermore, siltation enhances the habitats that promote water-borne disease vectors and siltation of reservoirs compromises water supply and hydroelectric power (HEP) generation (Boardman and Poesen 2006). Herein, it is clear that enhanced runoff and soil erosion have a major impact on food, water and energy security (Blake et al. 2018). Future land-management decisions require quantification of the anthropogenic amplification of runoff and soil erosion processes, e.g., sediment yield from different agricultural land uses, so that hotspots of soil erosion can be targeted and controlled specifically by mitigation measures.

Application of tools to support source apportionment should be grounded in a thorough understanding of hydrological and soil erosion processes (Morgan 2005) that lead to sediment generation from agricultural land for specific study sites. The mode of overland flow development, i.e., infiltration excess vs saturation overland flow can have a critical bearing on tracer behaviour in some circumstances and the particle size selectivity of soil erosion (Issa et al. 2006) can strongly influence source signatures.

Sediment dynamics in river systems involve complex processes and their quantitative assessment faces many uncertainties. River sediment budgeting approaches provide understanding of sediment mobilisation, transport, storage, and yield (Walling and Collins 2008), a useful framework within which to evaluate evidence on sediment and pollutant source dynamics. River sediment budgets can be understood, in simplistic terms, as the mass balance between the sediment sources, deposition areas, and outputs. One of the most significant findings from budgetary studies is the importance (and magnitude) of sediment storage in river systems. From the total amount of sediment produced (i.e., eroded) in upland surfaces (i.e., sources), only a fraction makes its way to the basin outlet (i.e., sediment yield). This discrepancy has been named the ‘sediment delivery problem’ (Walling 1983), and it has been argued that various sediment storage mechanisms operating within a catchment explain this discrepancy (Trimble 1983; Walling 1983; Fryirs 2013). Since then, increasing research has been carried out to explain and disentangle the mechanisms, pathways, and fates of eroded sediment within a river basin, and evidence on source dynamics is a crucial ingredient to support management decisions at the basin scale.

In many catchments, sediment spends significant time stored in riverine compartments which can compound challenges of source apportionment within specific time frames. Its delivery is therefore controlled by storage and sporadic remobilisation at various timescales (Muñoz-Arcos et al. 2022). These storage units can be defined as transient, short-lived landforms that are frequently reworked by episodic events such as bars, lateral deposits, and the streambed where they play a key role in the (dis)connectivity of catchment sediment cascades (Fryirs 2013). Spatial and

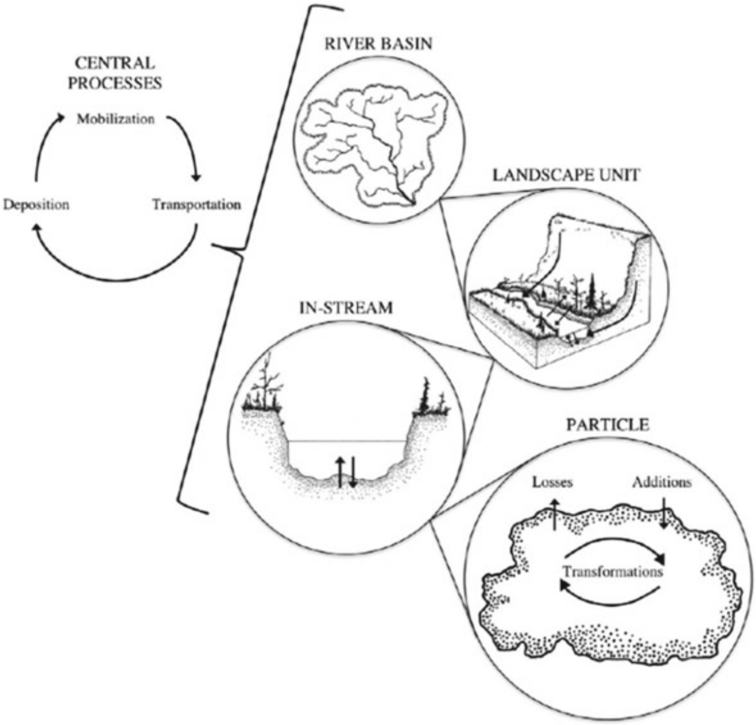

Fig. 7.1 Scales of complexity in sediment tracing applications (Koiter et al. 2013)

temporal aspects of sediment connectivity from hillslope to channel are important considerations in all source apportionment studies (Fig. 7.1).

Excess fine sediment (silt and clay fractions < 63 μm) poses a risk to receiving waters owing to both its physical presence as well as the potential for transport of co-contaminants. Many surface water systems are sensitive to changes in the transport and deposition of fine sediments owing to the impact of increased turbidity in the water column and siltation of channel bed habitats. For example, there is potential for siltation to affect oxygen demand and subsequently impact upon salmonid spawning grounds (Jensen et al. 2009; Sear et al. 2017). Excess suspended and stored sediment may also affect other, sessile biota such as the freshwater pearl mussel (*Margaritifera margaritifera*), which is critically endangered in Europe, according to the International Union for the Conservation of Nature (IUCN). Freshwater pearl mussels are sensitive to changes in water parameters, preferring oligotrophic and low turbidity conditions. For successful recruitment of juvenile freshwater pearl mussels, it is essential that the sediment in which juveniles establish has an open, well-oxygenated structure, enabling clear exchange between the interstices and the overlying water column. Excess fine sediment can negatively impact upon channel bed habitat by embedding coarse material and preventing exchange between interstices and surface (Geist and Auerswald 2007).

Transport of fine sediment from agricultural settings to receiving waters can also impact upon water quality through increased inputs of co-contaminants, which may exert direct or indirect effects depending on toxicity and bioaccumulations factors, and influence upon trophic structure. In relation to the latter, surface waters can be sensitive to elevated nutrient inputs associated with increased sedimentation, particularly with regard to phosphorus (P), often the growth-limiting nutrient in freshwater systems. Agricultural inputs can potentially increase the bioavailable pool of P, leading to eutrophication and associated ecological impacts (Withers and Jarvie 2008). Given that P is strongly associated with sediments, there is now a clear recognition of the need for improved management practices to reduce soil and associated phosphorus inputs to river channels (Ballantine et al. 2009; Ockenden et al. 2014). Significant improvements have been made to reduce the amount of dissolved P that enters watercourses from point source discharges across the EU. However, legacy sediment stored within channel and floodplain systems has the potential to act as a secondary source of P to the water column following disturbance (van der Perk et al. 2007) or in response to changes in condition of overlying waters (Jarvie et al. 2005b; Neal et al. 2012). Desorption and remobilisation processes are highly dependent upon environmental factors such as pH and oxidation–reduction conditions but, nevertheless, could offset the benefit of reductions in P inputs in the short term (Burns et al. 2015).

Sediment associated contaminants from agricultural settings are not limited to nutrients but can be wide ranging and related to biological (faecal) contamination, metals, and an array of organic compounds used in crops such as pesticides, herbicides and fungicides, and veterinary pharmaceutical treatments. Many organic compounds are highly particle reactive and persistent and are, thus, now restricted in their usage. However, the persistence of legacy inputs in sediment storage zones may present a continued threat to aquatic systems (Holden et al. 2017).

7.2 Approaches and Theory for Sediment Source Tracing

7.2.1 Core Principles of the Methodology

Among the sediment source tracing methods, the sediment fingerprinting approach has been widely used to apportion sediment sources at different landscapes and catchment characteristics (Walling 2005; Owens et al. 2016; Collins et al. 2017, 2020). Sediment fingerprinting approaches are based on identification of statistically significant differences in soil properties between target river basin sediment source areas. If signatures of downstream mixtures can also be derived, and the key assumption that signatures are not altered during hydrological and fluvial transport (i.e., conservatively), then qualitative or quantitative comparison between source and mixture

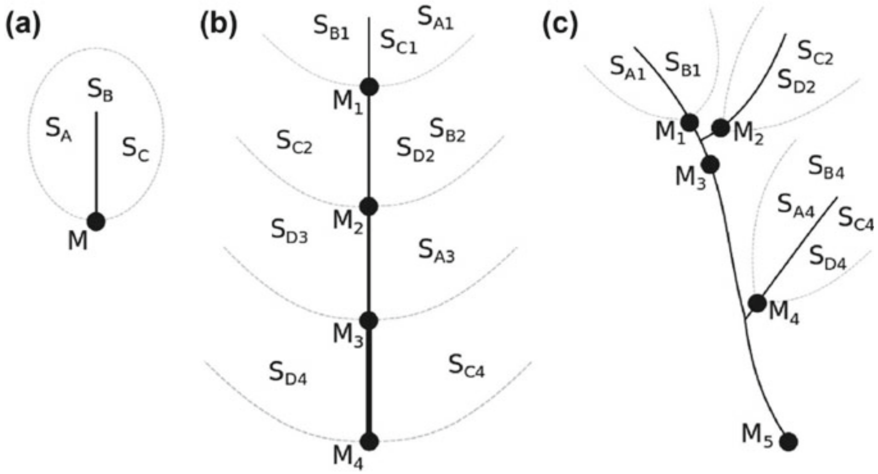

Fig. 7.2 Example experimental designs to link primary sources to sediment mixtures (Blake et al. 2018) where rivers flow downward, filled circles represent nodes at which sediment mixture (Mix = M) samples could be collected, and dashed grey lines delineate watersheds (S) denoted by subscript numbers, and subscript letters indicate unique sources. (A) Simple watershed with three sources, SA-C, and one mixture location at the outflow, M. (B) Longitudinal system with four sources, SA-D, and multiple mixture locations at the outlet of each nested subwatershed, M1–4. (C) Distributed system with mixtures at the outflow of each of three subwatersheds, M1, M2, and M4, four sources (SA-D), as well as mixtures on the main channel: M3 and M5. *Note* not all sources are present in all subwatersheds

fingerprints can elucidate dominant source contributions (Fig. 7.2). Differences in source properties and 'fingerprints' can occur due to a range of natural and anthropogenic processes generally linked to the geological substrate, cultivation practice, land use, and atmospheric depositions or pollution.

Properties that have been used to discriminate sources include: (i) fallout radionuclides (FRNs) (e.g., ^{137}Cs, ^{7}Be, and excess ^{210}Pb$_{xs}$ defined as the difference in activity concentrations between ^{214}Pb and total ^{210}Pb) which effectively label surface soil material permitting discrimination of surface, subsurface, and cultivated sources (e.g., Mabit et al. 2008; Wilkinson et al. 2009); (ii) major and minor element geochemistry which is related to geological substrate but is also modified by soil formation processes and weathering and therefore can discriminate between land use (e.g., Collins et al. 2010; Laceby and Olley 2015); (iii) Compound Specific Stable Isotopes (CSSIs) which are organic fingerprints related to soil plant cover (e.g., Blake et al. 2012; Bravo-Linares et al. 2018; Upadhayay et al. 2018b); (iv) mineral magnetic properties which are sensitive to soil formation processes and pollution (e.g., Ley and Devon 1998; Blake et al. 2006); (v) contaminants from industrial or other anthropogenic activities, e.g., heavy metals (e.g., Rothwell et al. 2005); and (vi) sediment colour (e.g., Pulley and Collins, 2021, Barthod et al. 2015).

Collins et al. (2020) provide comprehensive reviews of the wider methodology and key considerations in source apportionment applications for the user community.

Here, we focus specifically on practical application of techniques that fall within the 'nuclear tools' domain: fallout radionuclides (FRNs), X-ray fluorescence (XRF) elemental geochemistry, and Compound Specific Stable Isotopes (CSSI).

7.2.2 The 'Nuclear Tools' Tracer Toolkit

Fallout radionuclides (FRNs) are delivered to surface soil via wet and dry fallout mechanisms. The three FRNs applied in source apportionment studies are ^{137}Cs, ^{210}Pb, and ^{7}Be. Cs-137 is a legacy radioisotope derived from thermonuclear weapons testing in the twentieth century with most fallout occurring during the 1950s and 1960s, and locations affected by fallout from nuclear power plant accidents, notably the Chernobyl incident in the 1980s. Fallout ^{210}Pb is the unsupported component of total ^{210}Pb present in soil and sediment. The supported component is in equilibrium with its geogenic parents in the uranium series. The unsupported fallout component enters the atmosphere via diffusion of its intermediate parent ^{222}Rn from regional bedrock and weathered substrates. Be-7 is produced by cosmic ray spallation of oxygen and nitrogen in the upper atmosphere.

All three FRNs are delivered to the surface predominantly by rainfall with the assumption of instantaneous binding at the soil surface. As with all tracer tools, such assumptions need careful consideration and evaluation in the specific context of any study site. For example, FRNs have been shown to be preferentially associated with fine-grained materials (He and Walling 1997; Taylor et al. 2012) raising critical questions about size selectivity of transport and influence on concentration-based tracer signatures (Laceby et al. 2017a). The different half-lives of the three isotopes and hence residence time in the soil profile is a key controlling factor of their utility as source tracers. With its half-life of ~ 30 years, ^{137}Cs delivered to the soil profile are subjected to downward translocation by both bioturbation and geochemical diffusion and migration. Furthermore, ploughing of the soil surface mixes ^{137}Cs uniformly through the plough layer. Similarly, the ~ 22-year half-life of ^{210}Pb means its fallout component in surface soil is subject to similar processes although it is less geochemically mobile than ^{137}Cs, and it tends to have a shallower depth profile in the soil. Ploughing of course leads to uniform mixing as for ^{137}Cs. The short half-life of ^{7}Be, 53 days, means it has a uniquely different profile in the soil compared to the longer-lived FRNs. The FRN remains present in the uppermost millimetres of the soil since activity concentrations of material bioturbated are rendered below detection limits by radioactive decay. If a soil surface is ploughed, the ^{7}Be activity of the surface is diluted to below detection limits effectively resetting the surface signature. The ^{7}Be signature of the soil surface is also highly dependent on fallout dynamics linked to spatial and temporal rainfall patterns (Taylor et al. 2016).

There are two useful models of FRN-based source signatures. The first (Wallbrink and Murray 1993) was developed for uncultivated scenarios representing degraded land subject to sheet wash and incision processes (Fig. 7.3a). In this context, variability in the activity concentrations of ^{7}Be and ^{137}Cs of downstream sediment can be linked back to sediment production processes. The second (Walling and Woodward 1992) is based on typical agricultural conditions (Fig. 7.3b) with a mixed cultivated and uncultivated land cover allowing apportionment to land use and process drivers.

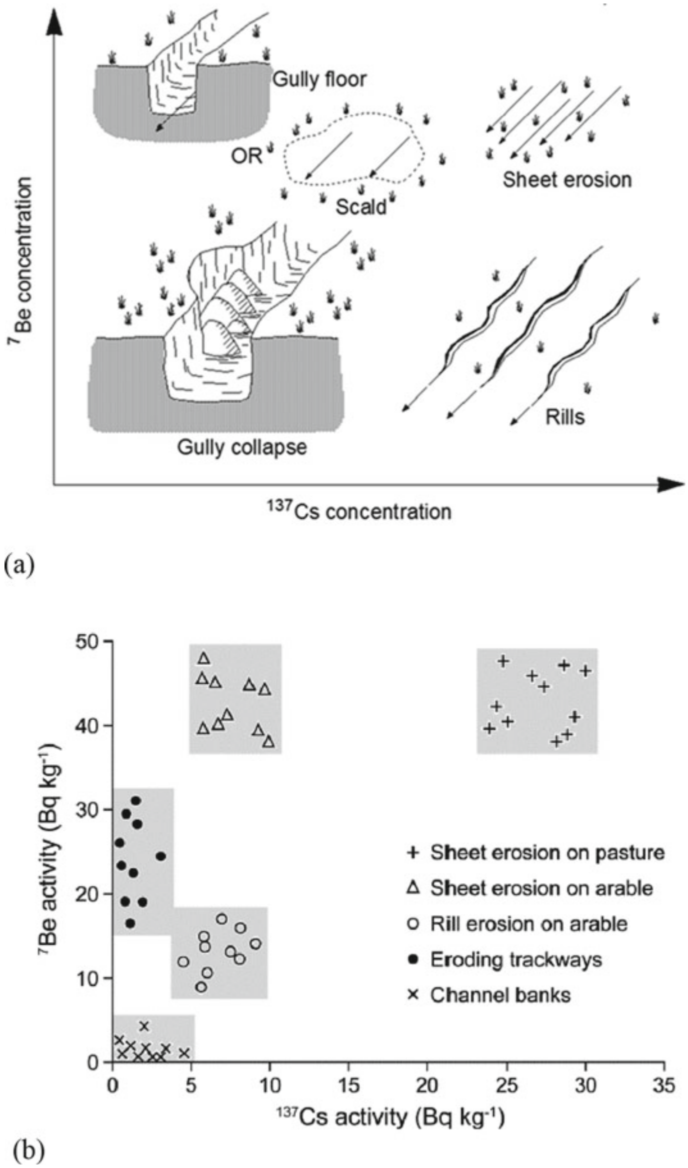

Fig. 7.3 Models of FRN application as a source tracers **a** for degraded landscapes subject to sheet wash and incision (Wallbrink and Murray 1993) and **b** agricultural settings with mixed cultivated and uncultivated land cover (Walling 2012)

XRF elemental geochemistry

Analysis of stable elements offers complementary data to CSSI and FRNs to provide multi-parameter datasets capable of improved source discrimination. Within a river catchment, source materials can be identified a priori based upon hydrological connectivity of land (source) units or via field observation. Element profiles may vary naturally in line with underlying geology or be altered by contaminant inputs such is the case for road-derived or mining-related materials for example. Agricultural practices can influence surface soil signatures through enhanced weathering of cultivated soils, such that differences between surface and subsurface element concentrations can often be detected. This may be further enhanced by atmospheric deposition at the soil surface (Smith and Blake 2014). Field survey sample numbers are dependent on the size of the study area, the range of potential sources, and gaining representative samples involves repeat sampling of each source type. Subsequently, tracing studies often require large sample numbers to be processed and characterised for element concentrations. The choice of analytical technique, therefore, requires careful consideration. Techniques such as inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) have the advantage of low detection limits ($\leq$ μg/kg), which offer potential for a wide range of tracers to be measured. However, these techniques measure elements in the aqueous phase, which requires the use of hazardous (e.g., strong acids) and time-consuming digestion methods prior to measurement. X-ray fluorescence (XRF) spectrometry offers an alternative and potentially rapid approach for measuring a broad range of elements in solid materials such as soils and sediments. XRF spectrometry provides total element concentrations and can, if required, be undertaken in a non-destructive manner on loose powder material, which is particularly useful where sample mass is limited. As well as laboratory-based instrumentation, the use of portable XRF instruments offers an opportunity for in situ screening of contaminant hotspots and source materials (Turner and Taylor 2018).

Compound Specific Stable Isotopes (CSSI) offer complementary/specific information to FRNs and geochemical approaches. Elemental geochemistry, fallout radionuclides, and mineral magnetism are sediment fingerprints that have been successfully applied to identify and apportion sediment sources within a catchment. However, they are limited when determining specific land use-derived sediment sources. The CSSI technique offers the possibility of refining other fingerprinting techniques by potentially allowing discrimination of different land uses based on the stable carbon isotopic composition of fatty acids $\delta(^{13}\mathrm{C})(_{\mathrm{FA}})$. CSSI refers to isotopes of individual compounds in complex mixtures rather than the isotopic signature of the bulk sample. Sources of different land uses can be identified based on stable isotope characteristics of specific molecules in vegetation communities. Soil contains a wide variety of organic compounds, which vary significantly in stability and structure, and therefore, the isotopic signature of a bulk soil is not expected to be conservative. Consequently, the need to target specific organic compounds appears. Plants synthesise complex organic molecules during photosynthesis using carbon dioxide. They can produce the same compounds (e.g., fatty acids) but with different isotopic compositions because of isotopic fractionation which will reflect the different photosynthetic pathways of the plants (Chikaraishi and Naraoka 2003; Reiffarth et al. 2016). Plant-derived FAs are incorporated into the soil through rhizodeposition and decomposition of organic matter transferring the $\delta(^{13}C)$ signature of the vegetation from which they are released. Fatty acids become bound to mineral and clay soil particles making them persistent for long periods of time (Gibbs 2008; Reiffarth et al. 2016; Upadhayay et al. 2017a). Although FA concentrations may change overtime due to degradation by microorganisms, volatilisation, dilution, and dispersion, they do not cause significant isotopic fractionation (Gibbs 2008). However, the use of the very long-chain fatty acids (VLCFAs) is particularly recommended for this purpose since they are predominantly plant-derived and more resistant to degradation in soils and sediments which avoids the influence of external sources such as contamination by microorganisms and algae and reduces the risk of isotopic fractionation (Reiffarth et al. 2016; Upadhayay et al. 2017b). Due to this fingerprinting method based upon qualitative data ($\delta(^{13}C)$(FA)), a further correction by the concentrations of each fatty acid in the source material considered in the fingerprinting approach is required (Upadhayay et al. 2018a). Different relative tracer concentrations in potential sources influence apportionment calculations, and the need to consider concentration-dependency in isotope mixing is strongly recommended (Upadhayay et al. 2018a).

7.2.3 Linking Source Materials to Downstream Mixtures

Quantitative comparison of source signatures to downstream mixtures is achieved through application of mixing models. With increasing attention to challenges of uncertainty and representativeness alongside the need to harmonised approaches, methodologies have emerged with exemplar structures and data to support user application. Recent innovations include application of MixSIAR (Stock et al. 2018) in river basin contexts (Blake et al. 2018); FingerPro purpose designed for the soil sediment continuum (Lizaga et al. 2020a), and the SIFT framework (Pulley and Collins 2018). Recent publications all provide code and /or open-source software to support user implementation. Nevertheless, a key element before performing any calculation is the careful choice of tracers (see also Chap. 3). Tracer selection is a crucial step for any fingerprinting study in which tracer conservativeness, source discrimination, and mixing model performance should be evaluated. Several strategies have been employed to select the most suitable tracers for sediment source apportionment such as boxplots, biplots, multivariate analyses, Kruskal–Wallis, and discriminant function analysis (DFA) tests, among others (Collins et al., 2020). For discussion about the performance of different tracer selection methods, the reader is directed to other works (Palazón and Navas 2017; Smith et al. 2018; Lizaga et al. 2020b).

7.3 Sample Preparation Techniques and Measurement

7.3.1 Understanding the Origin of Sediment Source Fingerprints

In all tracer applications, successful implementation must be grounded in a thorough process understanding of the origins of tracer properties and the expected differences between sources. A hypothesis-led approach to source characterisation is recommended to connect the study to established theoretical contexts but also, through the hypothesis testing process, to identify attributes that are unique to the study site in question.

For example, in the context of FRNs and questions of sediment contributions from different land use within the catchment, the starting point is to hypothesise the relative proportions of the three FRNs in each land use based on local environmental knowledge of catchment land use history over recent decades and months. A strategic sample design programme can then be implemented to evaluate consistency of signature within source type areas and confirm differences between types (Fig. 7.3).

In the context of XRF geochemical signatures, a useful starting point is the consideration of the broadest control on differences within the local geology, i.e., weathering profiles and surface versus subsurface material. From here the influence of land use can be hypothesised, e.g., mixing of soil by ploughing to create new signatures and/or amendment by agrochemicals.

In the context of CSSI applications, source samples are targeted to those representing the major land uses in the studied catchment. Sampling should focus mainly on those land uses which are supposed to be susceptible to erosion. To account for spatial variability in $\delta(^{13}\mathrm{C})_{(\mathrm{FA})}$ values, the collection of enough replicates of every source is recommended to properly represent the land use. The CSSI sampling is focused on the uppermost soil layers as this layer is the most susceptible to erosion. Therefore, the sampling depth is usually 20 mm, i.e., the soil layer in which most of the organic matter is found. In some cases, for assessment of gully erosion, road erosion, and riverbank erosion, the sampling could involve also much deeper layers.

A sampling programme that represents all potential sources is of course the goal in any study, but users must also be aware that a missing source will not be identified by the mixing model—the model will always report to a sum of unity whether all sources have been represented by sampling or not.

7.3.2 Sampling Strategies

Sampling strategies should naturally be adapted to the specific challenges of each scale of study (Fig. 7.1). With increasing spatial scale comes increased field sampling, processing and analytical demand. Herein, it is useful to consider two broad approaches to source definition that can be tailored to catchment-specific sample designs.

The *primary source approach* (Fig. 7.2) is a straightforward characterisation of each primary source within the system of interest and comparison to mixtures downstream in lower-order channels. The use of spatially integrated composite samples from primary sources is widely recognised as a means to ensure representative characterisation of properties. For example, one sample might comprise 10–30 smaller samples taken from a designated area within the source zone. Replication is essential, and the user must satisfy themselves that the number of samples for a source classification is both environmentally and statistically representative to permit effective source discrimination. At the large catchment to basin scale, characterisation of all primary sources can become unwieldy and stretching resources as well as introduce complexity in unmixing model development. In this case, a *tributary approach* might prove useful where the mixtures in higher-order channels or receiving water bodies are compared back to the sediment mixtures in transit or storage in lower-order tributaries upstream (see also Chap. 2). The advantage of the approach is that hydrological processes above the tributary source end member will have created a spatially, and potentially temporally, integrated sample at the subcatchment outlet. A disadvantage is where management practice and erosion response within the lower-order tributary catchment areas are heterogeneous and complex. A hybrid model might use specific tributaries of known land use and/or source type as representative of a specific source end member, assuming other controls on soil properties e.g., geology are uniform. These challenges are explored in the case studies (Sect. 7.4).

7.3.3 Laboratory Analysis

Measuring FRNs via gamma spectrometry

Detail on gamma spectroscopic methods to determine FRN activity concentrations in soils and sediment is provided in detail by Iurian and Millward (2019). For all tracer applications, users need to carefully consider the particle size fraction that they wish to compare between source and sink (Laceby et al. 2017). Critically, one must compare like with like and further evaluate particle size assemblages to verify.

Once screened to the relevant particle size (e.g., $< 63\ \mu m$) samples must be sealed in gas-tight and stored for 21 days to permit equilibration between ^{214}Pb and its parent radioisotope ^{226}Ra prior to measurement by gamma spectrometry (Appleby 2001). Activity concentrations of the target radionuclides are measured using a low background HPGe Gamma spectrometry system. Instruments should be calibrated using soil material spiked with certified mixed radioactive standards supplied by a certified provider. All calibration relationships are generally derived using proprietary software and should be verified by inter-laboratory comparison tests, e.g., with reference materials supplied by the IAEA via worldwide proficiency tests. Total ^{210}Pb is measured by its gamma emissions at 46.5 keV and its unsupported component calculated by subtraction of ^{226}Ra activity, which in turn was measured by the gamma emissions of ^{214}Pb at 295 and 352 keV. ^{137}Cs is determined by its gamma emissions at 662 keV (with correction for ^{214}Bi emissions). Short-lived ^{7}Be is analysed via its emission at 477 keV. Count times typically range 24–48 h subject to activity concentrations.

XRF spectrometry for elemental geochemistry

X-ray fluorescence (XRF) spectroscopy enables total element concentrations to be determined without the need for time-consuming and hazardous digestion procedures and has, thus, been widely applied in aquatic and marine contaminant research. XRF spectrometry is often employed as a complementary analytical technique in tracing studies with the potential to provide data for a wide range of elements (typically Na to U) at mg/kg concentrations. There are two major branches of XRF spectrometry: energy dispersive XRF (ED XRF) and wavelength dispersive XRF (WD XRF). Both techniques employ the same fundamental principles of ionisation in the sample material although the techniques differ in their dispersion and detection of secondary X-rays. WD XRF spectrometers provide the advantage of improved resolution and sensitivity for low energy elements (particularly Na to P), whereas the simultaneous nature of ED XRF spectra analyses provides a time advantage over WD XRF (the latter typically employing sequential detection of elements). Despite the differing detection methods, sample matrix and the manner in which the sample is presented to the spectrometer are equally important to both techniques. This is particularly relevant for the analysis of trace elements in sediments, which are often analysed as pressed powder pellets. Sample homogeneity is a key consideration for the operator, and it is crucial that the sample preparation ensures that particle size in the sample is uniform and below ~ 50 μm to reduce particle size and shadowing effects in the analysis layer (Willis et al. 2011). If the particle composition is also heterogeneous, such that an analyte is present in more than one mineral form, mineralogical effects can occur whereby the intensity of the analyte line is affected by differing attenuation properties of the particles. These matrix effects can only be eliminated by fusion methods, which involve mixing the sample with a flux material, typically lithium tetra/metaborate, at high temperature. The resulting glass disc provides a homogenous geometry for analysis (Willis et al. 2014). Owing to the flux to sample ratios used in the fusion process, XRF analysis of fused materials is usually limited to major elements, which will remain detectable even when diluted by the addition of flux.

Sample preparation and extraction for CSSI signatures

Fatty acid (FA) extraction is the first key step in the process. Several methods can satisfactorily extract FAs, namely Accelerated Solvent Extraction (ASE); Microwave-Assisted Extraction (MAE); Ultrasonic-Assisted Extraction (UAE) and Soxhlet. A detailed description and assessment of the performance, advantages and disadvantages of these methods can be found elsewhere (Jeannotte et al. 2008; Hewavitharana et al. 2020). FAs bound to the soil matrix can be extracted using hot/pressurised nonpolar solvents such as dichloromethane (DCM), or a mixture of solvents (e.g., DCM/Methanol) depending on the extraction method used. Samples must be dried before performing any extraction. This can be done by drying at 60 °C in a laboratory oven or using a freeze-dryer. Drying with anhydrous sulphate in direct contact with the wet sample or air-drying is also an alternative when no instruments are available. When using Soxhlet, soil, and sediment samples plus a pinch of anhydrous Na_2SO_4 (used to remove sample moisture) are put in cellulose filter containers, about 250–300 mL of DCM is added to each Soxhlet balloon, and heaters are turned on keeping temperature at 40 °C. The solvent will evaporate and condense in the reflux flask, drip onto the filter container and then the extract is collected into a round-bottom flask. When using ASE, soil and sediment samples are placed in stainless-steel cells and a proper extraction programme is selected. The DCM is injected into the cell, and the sample is heated and pressurised with N_2 (at 100 °C and 2000 PSI). The sample extract is then collected in a sealed vial. For MAE, samples are placed in extraction chambers, and depending on the sample amount, DCM:Metanol (4:1) solvent mixture is added and a proper extraction programme is selected (combining temperature, time and power). When using UAE, samples are transferred into Schott Duran flasks. The sample (10–20 g of dry sample) is then soaked in 100 mL of DCM and placed in an ultrasonic bath for at least 30 min. For both, MAE and UAE, samples must be filtered after the extraction process to remove particles using a suitable filter paper (e.g., Grade 1 Whatman). In addition, it is suggested that the extraction procedure for both (MAE and UAE) can be performed twice to assure quantitative (i.e., complete) extraction of the analytes. In this case, filtrates are combined. To reduce the solvent volume in the solution, a rotary evaporator is used. The temperature is set accordingly (around 40 °C depending on the solvent used), and the reduction process is interrupted when 2–5 mL of the solution remains in the rounded flask. The concentrated sample is then quantitatively transferred to a 10 mL vial using a Pasteur pipette. The vial is then inserted into a water bath at 40 °C, and the extract is subjected to a current of ultrapure N_2 to complete dryness.

Fatty acid derivatisation is the next step. Derivatisation for gas chromatography (GC) analysis is performed to increase volatility, improve separation and reduce tailing of the analytes. Because FAs have high polarity and low volatility, derivatisation is highly recommended (Brondz 2002). In this process, the polar carboxylic acid is converted to a methyl ester. The derivatisation process (i.e., esterification) results in a less polar molecule called Fatty Acid Methyl Ester (FAME). Although, there are several methods reported in the literature for FA derivatisation with reproducible results (Brondz 2002), the method described below is based on the use of methanol as derivatisation agent catalysed with boron trifluoride (BF_3). It is essential to remember that a portion of the methanol used during the derivatisation process must also be sent to the analytical laboratory to obtain the carbon isotopic value of the added methyl group. This will be further used to correct the isotopic values of the FAMEs. It is advisable that all reagents should be prepared daily before starting the derivatisation procedure. To the dry extract, 1 mL of 5% of BF_3 in methanol (BF_3-MeOH) is added and mixed on vortex for 2 min. The samples are then placed in a test tube rack in a fan oven/block heater at 70 °C for 20 min and cool down at room temperature. Then, 1 mL distilled water and 1 mL of hexane/DCM (4:1) mixture are added to the samples and mixed on vortex for 1 min. Posteriorly, samples are let to stand to allow the solvent layers to separate. Carefully, the upper layer (organic phase) is transferred from the 10 mL screw cap test tube to a 2 mL vial by using a Pasteur pipette. The procedure is repeated, but this time adding only 1 mL of hexane/DCM mixture and transferring the upper layer again. At the end, the vial will contain about 2 mL of the FAME extract. It must be stored at 4 °C until analysis or fully dried with ultrapure N_2 if samples are being analysed in an external laboratory.

The $\delta(^{13}\mathrm{C})$ values of the FAMEs are adjusted using the following formula to account for the addition of the methyl group added (Gibbs 2008):

$$\delta(^{13})C_{\mathrm{FA}} = \frac{\delta(^{13}C)_{\mathrm{FAME}} - (1 - X)\delta(^{13}C)_{\mathrm{methanol}}}{X},$$

where X is the fractional contribution of the free fatty acid to the methyl ester (e.g., 22/(22 + 1) for a FA of 22 carbons); $\delta(^{13}\mathrm{C})_{\mathrm{methanol}}$ is the isotopic composition of the methanol used during the derivatisation process; $\delta(^{13}\mathrm{C})_{\mathrm{FA}}$ refers to the corrected isotopic composition of the FA, and $\delta(^{13}\mathrm{C})_{\mathrm{FAME}}$ is the isotopic composition of the FAME provided by the instrument.

IRMS analysis for CSSI

A gas chromatography-combustion-isotope ratio mass spectrometer (GC-C-IRMS) is used to analyse the $\delta(^{13}\mathrm{C})$ value of fatty acid esters. Samples are injected into the gas chromatograph. Then the sample is vaporised at 280 °C. Thus, the analysed samples in a GC-C-IRMS system must be volatile. Subsequently, the FAMEs are pushed with ultrapure helium gas to pass through the chromatographic column, whose function is to separate them by molecular weight/boiling point and affinity with the stationary phase of the GC column.

From the GC column, the FAMEs go to the combustion reactor, whose function is to convert the compounds into CO_2 and H_2O. This reactor consists of an Alumina tube (Al_2O_3) at 1000 °C. Its internal part contains a wire (composed of copper, nickel, and platinum) that is purged with oxygen allowing oxidative combustion, where nickel acts as a catalyst. The combustion products pass through a moisture trap, where the water present in the gas mixture is removed. This trap consists of a selectively permeable membrane with a dry helium counterflow. The resulting CO_2 is directed to the IRMS device and $\delta(^{13}\mathrm{C})$ for each fatty acid can be obtained. From an isotope ratio mass spectrometer (IRMS), it is possible to measure isotopic composition at low enrichment and natural abundance levels. It means that low variations in exceedingly small amounts of the heavier (or less abundant) isotope are detected in the presence of large amounts of the lighter isotope with high precision and accuracy.

Particle size analysis

Tracers often display preferential association with particle size classes in source and channel material, with enrichment in finer particle sizes common for many elements and FRNs (Taylor et al. 2014; Laceby et al. 2017b). Where particle sorting occurs during sediment transport and storage, it is important for the user to account for particle enrichment during tracing studies. Many studies account for sorting effects by directly analysing transported size fractions in both the source and stored sediment material. Other studies apply particle size correction factors based upon empirically derived relationships between particle size and tracer concentrations (Smith and Blake 2014; Laceby et al. 2017a). Particle size analyses of source and transported material may, therefore, be required. The traditional pipette method is based upon the use of a settling column and Stokes' Law to determine particle size by subsampling the suspension at a predefined depth and time. A more contemporary approach combines the use of Stokes' law with a paralleled X-ray beam to determine sediment concentration in the settling column (SediGraph). Laser diffraction is a commonly applied approach for particle size analysis offering an effective means of analysing large sample numbers and has been shown to provide good agreement with digital image analysis (Bittelli et al. 2019).

7.4 Applying Nuclear Tools for Tracking Sediment Pollution from Agriculture (Case Study)

7.4.1 Applying FRNs to Identify Agricultural Runoff Source and Connectivity

Context

The River Mease, located in central UK, is a mixed agricultural river basin in a temperate maritime climate. The land use comprises a range of cultivated areas growing fodder maize, potatoes and cereal crops with areas of pasture for livestock grazing where field sizes are of the order of 5–15 ha. The topography is gently undulating with low to moderate slopes. At the time of study (Blake et al. 2013), the main river system was classified as being in an 'unfavourable condition' for aquatic habitat with respect to high silt and phosphorus concentrations in the riverbed substrate.

Sediment and associated diffuse phosphorus pollution from agriculture were considered to be significant contributors to unfavourable condition. Water quality modelling had suggested that in the region of 40 % of dissolved phosphorous, inputs to the catchment were derived from diffuse sources, with agricultural sources likely to be the main contributor. In parallel, sewage treatment works and domestic septic tanks had also been identified as potentially important sources of phosphorus, wherein the former was being tackled with improvements to phosphorus removal. The debate surrounding agricultural versus domestic sources of phosphorus was an important management question but was constrained by evidence being limited to phosphorus in the dissolved phase. Given that the greatest proportion of phosphorus delivered from hill slope to channel is in particulate form (Owens and Walling 2002) and potentially exchangeable with surface water once in aquatic storage (Jarvie et al.

2005a, b), a key research question arose regarding sediment sources in relation to agricultural activities.

Sampling and analysis

To characterise source materials, ten spatially integrated samples were collected from representative areas of each primary source type that were relevant to FRN characterisation. These were cultivated soil, uncultivated soil, subsurface drains, soil material deposited on roads within the agricultural areas (considered as secondary sources) and stream channel banks.

To characterise sediment mixtures, samples of sediment stored within the channel bed matrix of six tributaries were collected in triplicate using a stilling well methodology early in the winter 'wet season' period. Suspended sediment samples were collected via time-integrated samplers for early and later stage rainstorm events during the main winter period. All samples were freeze-dried and gently disaggregated and sieved to $< 63\ \mu$m. Samples were sealed into 50 mm Petri dishes and stored for 21 days to permit equilibration between ^{214}Pb and its parent radioisotope ^{226}Ra prior to measurement by gamma spectrometry (Appleby 2001). Activity concentrations of the target radionuclides were measured using a low background EG&G Ortec planar (GEM-FX8530-S; N-type) HPGe gamma spectrometry system at the University of Plymouth Consolidated Radio-isotope Facility. The instrument was calibrated using soil material spiked with certified mixed radioactive standards supplied by AEA Technology Plc. All calibration relationships were derived using EG&G Gamma Vision software and verified by inter-laboratory comparison tests with materials supplied by the IAEA, namely the worldwide proficiency test using moss soil (IAEA-CU-2009-03). Total ^{210}Pb was measured by its gamma emissions at 46.5 keV and its unsupported component calculated by subtraction of ^{226}Ra activity, which in turn was measured by the gamma emissions of ^{214}Pb at 295 and 352 keV. ^{137}Cs was determined by its gamma emissions at 662 keV (with correction for ^{214}Bi emissions). An additional element to this study was analysis for short-lived ^{7}Be, analysed via its emission at 477 keV. Count times were typically 48 h due to low activity concentrations and low sediment sample mass.

Results and discussion

The three fallout radionuclides measured provided important information on sediment source dynamics. ^{7}Be concentrations were observed to be high in all surface soil samples. The other FRN signatures of cultivated and uncultivated surface soil showed a pattern seen typically in previous UK studies (Walling and Woodward 1992) whereby uncultivated soils carried higher activity concentrations of ^{137}Cs and ^{210}Pb$_{xs}$ due to the shallow depth profiles of both radionuclides in undisturbed soil. Cultivated soil represents a mix of the surface and shallow subsurface soil materials which effectively lowers the activity concentrations of both FRNs. Channel banks materials had measurable amounts of ^{137}Cs and ^{210}Pb, but lower than surface soils but *undetectable* activity concentrations of ^{7}Be. Material from field drain excavations had very low FRN concentrations across all three FRNs. The road-transported sediment in agricultural areas exhibited the greatest ^{210}Pb$_{xs}$ activity concentration of all materials sampled. These high activities were likely due to scavenging of $^{210}\mathrm{Pb}_{\mathrm{xs}}$ by soil in transit from surface pluvial runoff on the impervious surface (i.e., binding to suspended sediment in preference to the surface) and high particulate-water interaction time (Charlesworth and Foster 2005). Given that the road dust samples were collected from rural lanes at the bridging points where channel access was gained, they are highly representative of material transported to the channel via the road network. The material itself was likely to comprise sediment transported by run-on from agricultural fields, the FRN signature of which is transformed by $^{210}\mathrm{Pb}_{\mathrm{xs}}$ enhancement as above.

The FRN activity concentrations (Table 7.1) of sediment mixtures in the six tributaries showed notable differences between the channel-stored sediment and suspended sediment in terms of $^{7}\mathrm{Be}$ concentration. The predominant undetectable-to-low activity concentrations of $^{7}\mathrm{Be}$ in the channel-stored sediment sampled at the beginning of the wet season indicated either a long residence time (i.e., greater than 5 half-lives, ~ 250 days) or a predominant channel bank source. The activity concentrations of $^{137}\mathrm{Cs}$ and $^{210}\mathrm{Pb}$ of these samples were within the bank to cultivated land zone (see Fig. 7.4). The switch to $^{7}\mathrm{Be}$ rich material in most of the suspended sediment material collected indicated first, and most importantly, that a large proportion of the material in transit through the wet season to January was freshly eroded from an exposed soil surface, although there was a wide range in these values. This range could have reflected varying contributions of remobilised 'old' sediment from the bed or contributions from subsurface and bank sources (which have been shown to be deficient in $^{7}\mathrm{Be}$). The suspended sediment sample from catchment 2 was an exception, with undetectable $^{137}\mathrm{Cs}$ and low $^{7}\mathrm{Be}$ suggesting a substantial contribution was made from a subsurface source, whereas the other samples from this time indicated surface soil with varying contributions from cultivated and uncultivated land. In the later period, this system was more in line with the other sites in terms of freshly eroded material in transit. It was notable that the three catchments with the highest $^{7}\mathrm{Be}$ content in the second set are the three that had the greatest particulate phosphorus concentrations again implying fresh sediment delivery from intensively farmed land. Across all suspended sediment samples, $^{7}\mathrm{Be}$ showed a weak but positive correlation ($r = 0.33$), with particulate phosphorus which strengthened markedly ($r = 0.8$) when low particulate phosphorus outliers from catchments 1 and 5 were not considered. The close affiliation of $^{7}\mathrm{Be}$, indicating recent surface erosion, and particulate phosphorous concentrations in catchments 2, 3, 4, and 6 suggested mobilisation of soil from intensively farmed land that was well connected to the stream network via roads (linked to enhanced $^{210}\mathrm{Pb}_{\mathrm{xs}}$).

The indication from the FRN data is that the material stored in the subcatchment channels has a high residence time (deficient in $^{7}\mathrm{Be}$ cf Wilson et al. 2007) with activity concentrations of other FRNs in line with catchment soil sources. While this material might comprise bank slumped material, which has been shown to also carry FRN signals similar to cultivated land in this system due to exposure in the past or banks comprising reworked catchment materials, there was little evidence of bank erosion in the field.

Table 7.1 FRN activity concentrations (Bq kg^{-1}) of channel-stored and suspended sediments sampled in phase 2

Subcatchment	^{137}Cs	±	^{210}Pb	±	^{7}Be	±
Bed sediment early wet season						
1	4	*2*	34	*19*	< 14.99	
2	5	*2*	29	*17*	< 48.6	
3	6	*2*	44	*18*	< 63.0	
4	5	*2*	60	*25*	85	*50*
5	< 2.49		37	*21*	< 42.7	
6	7	*1*	57	*17*	79	*28*
Suspended sediment early wet season						
1	7	*2*	24	*17*	93	*30*
2	< 0.91		10	*6*	38	*19*
3	8	*2*	46	*18*	135	*40*
4	4	*1*	85	*18*	251	*45*
5	7	*3*	92	*32*	178	*62*
6	4	*1*	41	*14*	82	*38*
Suspended sediment late wet season						
1	11	*4*	55	*28*	164	*73*
2	5	*2*	52	*26*	147	*41*
3	11	*4*	80	*37*	251	*87*
4	7	*2*	77	*27*	257	*51*
5	7	*2*	44	*17*	84	*26*
6	8	*2*	50	*23*	148	*39*

The ^{7}Be content and particulate phosphorus loading of the suspended material collected during the major storm events, along with the other FRN signals, demonstrated a substantial contribution from surface soils in catchments 2, 3, 4, and 6, wherein roads were an important connectivity pathway linking the agricultural runoff, containing sediment and phosphorus pollution, to the rivers.

7.4.2 Applying XRF Signatures to Evaluate Contribution of Agricultural Runoff to Aquatic Habitat Siltation

Context

Freshwater Pearl Mussels (FWPM), *M. margaritifera*, are among the most critically threatened freshwater bivalves worldwide. In addition to their important roles in particle processing, nutrient release, and sediment mixing, they also serve as an ideal target species for evaluation of aquatic ecosystem functioning especially of

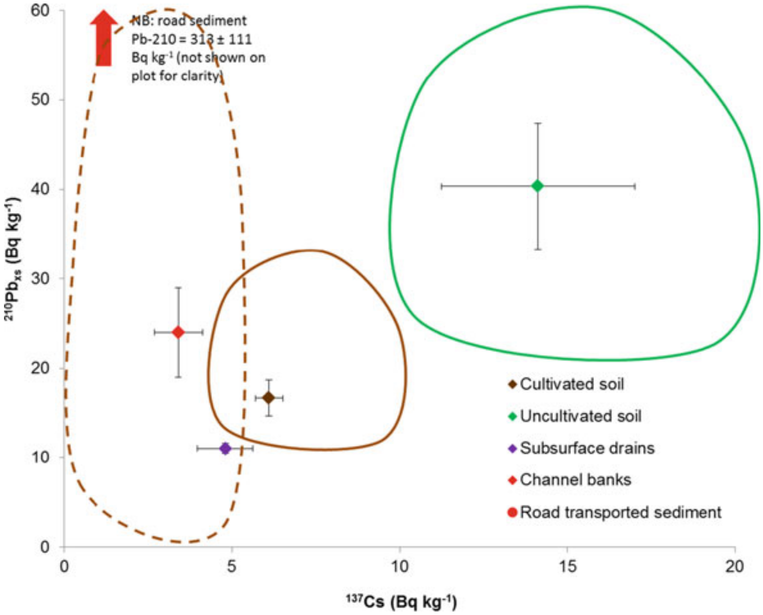

Fig. 7.4 FRN signatures for primary source materials where outlines represent range extent and uncertainty is one standard deviation of the mean, shown by the symbols

their symbiotic relationship with Atlantic salmon *Salmo salar* and brown or sea trout *Salmo trutta*. Poor water quality, particularly eutrophication, and siltation are considered major contributory factors in the decline of the species; hence, management of diffuse water pollution from agriculture is a key priority in catchments that host FWPM habitats. The river Clun, UK, is one such system where FWPM have been identified to be under threat (Blake et al. 2016).

FWPM populations have declined dramatically throughout its range in Europe and North America, and recruitment of juvenile mussels only occurs in a small fraction of the streams where populations still persist. There are a range of environmental threats FWPM populations linked to direct pollution and damage to habitat. Indeed, there had been a decline of more than 90 % in European populations by the 1990s, and it is accepted that this has continued or even increased since (Geist and Auerswald 2007). Currently, the main concern for FWPM populations in general is that juveniles do not seem to be reproducing, so populations are becoming more elderly. Poor water quality, particularly eutrophication, and siltation are considered major contributory factors in the decline of the species; hence, management of diffuse water pollution from agriculture is a key priority in catchments that host FWPM habitats.

The River Clun is located in central UK and drains a catchment 272 km^2. The principal land use within the catchment is sheep and cattle grazing although there are notable areas of cultivated land. In the lower reaches, the river is protected as a Special Area of Conservation (SA-C) and Site of Special Scientific Interest (SSSI) due to the presence of one of the few lowland populations of FWPM in the UK. At the time of study, adult FWMPs were reported to be reproducing in the Clun since glochida had been found on fish but juvenile/young adult FWPMs were, however, generally absent in surveys leading to the hypothesis that they were not surviving in the channel bed substrate to emerge as adults. It was hypothesised that siltation was a key factor so sediment fingerprinting tools were applied to identify the domain sources of polluting sediment.

Sampling and analysis

The sediment fingerprinting approach was applied at two levels in the study basin to determine the principal sources of fine sediment present in the failing FWPM beds in the higher-order channel.

Firstly, at the broad spatial scale, the main tributaries (Fig. 7.5) were treated as integrated source end members and a fingerprinting approach applied through analysis of fine sediment captured and stored in the channel at the stream outlets. This was then compared to material stored in the FWPM reaches. Importantly, this framework also included mainstem channel banks as a potential sediment source. Therefore, this approach allowed the identification of the relative contribution from the different tributaries and mainstem bank erosion to the sediment found within the FWPM beds.

Secondly, the subcatchment scale phase of the sediment fingerprinting attempted to compare sediment collected from the tributary outlets to primary sources in the wider catchment namely: (i) cultivated soil, (ii) uncultivated soil, (iii) channel bank

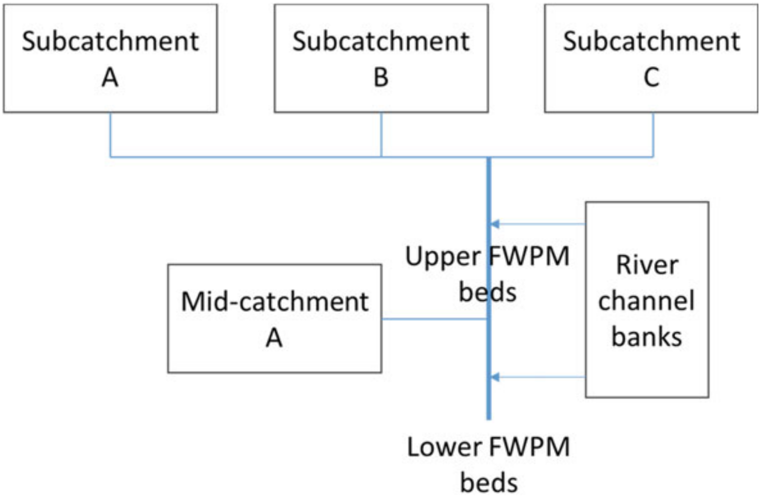

Fig. 7.5 Schematic of subcatchment scale experimental design showing link between target FWPM reaches on the main channel and subcatchment areas sampled using stream outlet sediment (providing an integrated source signature)

erosion, (iv) farm tracks, (v) road verges, and (vi) road-transported material. Therefore, this approach allowed the identification of the relative contribution from the different sources to the sediment found at the tributary outlets.

Using these two sets of results allows the identification of the tributaries that are significant sources of sediment to the FWPM beds and, within those tributaries, the identification of the primary sources that are most significant.

Channel-stored sediment and suspended sediment were collected in triplicate from the outlet of each tributary. The stilling well method (Lambert and Walling 1988) was used to recover fine sediment stored on and within the uppermost part of the channel bed matrix that was disturbed by water column agitation. Samples were collected during each quarter of the project to characterise spring, summer, autumn, and winter conditions. Suspended sediment was collected using time-integrated samplers (Phillips et al. 2000). These were installed to collect summer (drier season) and winter (wetter season) sample sets. Sampling at the FWPM beds was restricted due to ecological sensitivity of the area. The suspended sediment traps were installed away from FWPM areas but in the representative reaches.

Samples were freeze-dried, disaggregated and sieved to $< 63\ \mu\text{m}$ to ensure source and mixtures were like-for-like comparison. Elemental geochemistry was analysed via WD XRF using a PanAlytical Axios Max instrument (Sect. 7.4.2). Samples were also analysed for FRNs as described in Sect. 7.4.1. Geochemical unmixing for source apportionment was undertaken using the MixSIAR model as described by Blake et al. (2018).

7.5 Results and Discussion

Owing to the ubiquitous nature of the FRN signal across the catchment, these data (Fig. 7.6) provided a broad framework for interpretation of the geochemical unmixing results.

The FRN signatures of primary source material (i.e., cultivated soil, uncultivated soil, channel bank, and road-transported/derived material) conformed to established knowledge based on previous UK studies (Walling and Woodward 1992) wherein uncultivated soils carried higher activity concentrations of ^{137}Cs and ^{210}Pb$_{xs}$ due to the shallow depth profiles of both radionuclides in undisturbed soil (Figs. 7.7 and 7.8). Cultivated soil was a mix of the surface and shallow subsurface soil materials which effectively lowers the activity concentrations of both FRNs. Channel bank material was deficient in both FRNs due to subsurface nature of material, i.e., much of the channel bank is not labelled by these FRNs. There was also a notable activity concentration of ^{137}Cs measured in road sweepings with enhanced ^{210}Pb activity concentrations where interaction of pluvial, i.e., surface flow after rainfall, water with fine-grained material offered opportunity for enrichment. In this regard, it is important to also note the elevated ^{210}Pb$_{xs}$ activity concentration of road material which was enhanced compared to surface soil. These observations along with the range of ^{137}Cs concentrations of road sweepings ranging from uncultivated to cultivated soil imply

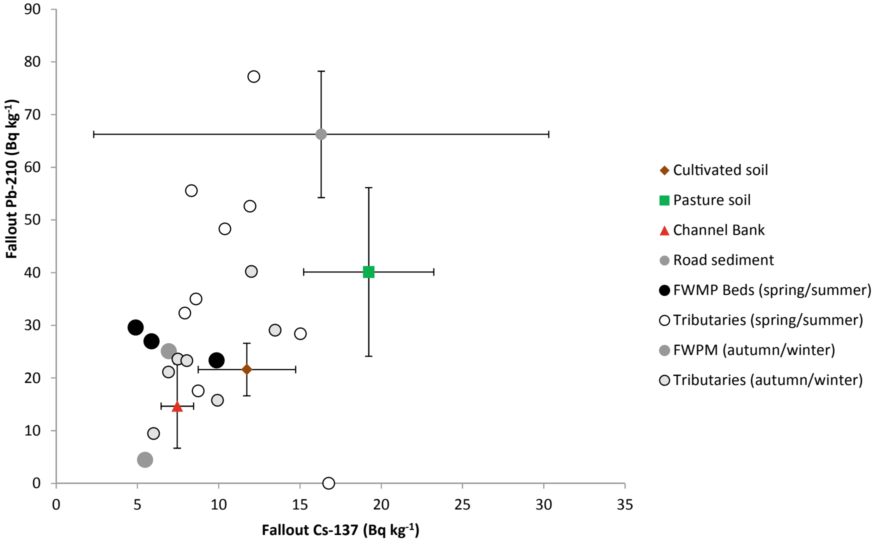

Fig. 7.6 FRN signatures for sources and mixtures samples from both tributaries and the high-order river channel

that the soil material sampled from roads originated from surface sources but was subsequently enhanced in $^{210}Pb_{xs}$ scavenged from pluvial road runoff water, i.e., tracer fingerprints developed as a consequence of the delivery pathway (Belmont et al. 2014).

Comparison of channel sediment FRN signatures to source materials offers insight into the different nature of main channel sediment compared to tributary sediment. The FRN signals of all material analysed from the main channel reach that hosted the FWMP beds (both upper and lower areas) were suppressed in terms of ^{137}Cs and $^{210}Pb_{xs}$ content and plotted in the range of channel bank material and cultivated material (Fig. 7.8). This suggests channel bank erosion was an important contributor to sediment sampled from the FWPM beds. The tributary sediment also showed an influence of channel bank erosion but the wider spread of FRN signals indicates that in the subcatchments, other sources were also important. Most tributary sediments had a ^{137}Cs concentration in the cultivated soils range with some elevated $^{210}Pb_{xs}$ concentrations although another possible permutation of sources that could lead to this is a combination of pasture and channel bank erosion. There was a general shift towards the lower corner of the FRN bi-plot space (Fig. 7.6) in the autumn/winter set indicating greater subsurface erosion (channel bank) in the more hydrologically active months.

Whichever the main control on surface soil inputs, the FRN data were unequivocal that channel bank erosion was a major sediment source in this system and that the FWPM beds in the main channel of the Clun contained a significant amount of sediment from this source.

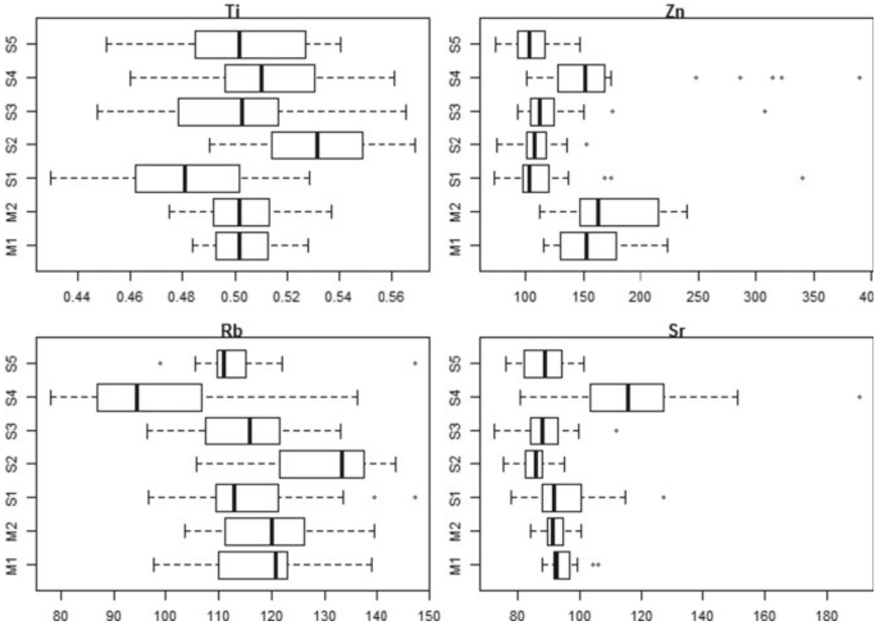

Fig. 7.7 Illustrative examples of tracer boxplots comparing source term range (S1, S2…) to mixture range (M1, M2) where Zn demonstrates non-conservative tracer behaviour

Geochemical data (Fig. 7.7) were analysed in the context of broader FRN interpretations. Tracers for application in the mixing model were selected using exploratory data analysis tools (Blake et al. 2018) with elements (examples in Fig. 7.8) that appeared to show non-conservative behaviour (e.g., Zn concentration of suspended sediment falling outside the range of the source materials) being removed from analysis. The FRN inferences of a strong influence of cultivated soil in subcatchments, with a road-sediment signal indicating conveyance path and influence of bank erosion in main channel sediments, were supported by geochemical fingerprinting data (Fig. 7.7). These demonstrated cultivated soil transported by road as a key factor in the most intensively farmed subcatchments (Fig. 7.8 left). While the main channel also showed a strong cultivated source signal in spring–summer, winter signals were dominated by channel bank erosion inputs (Fig. 7.8 right) which is in line with visual evidence of bank collapse on the main channel reaches harbouring FWMPs. While catchment-wide diffuse water pollution from agriculture in the form of sediment input was indicated, and roads provided an efficient transport vector, the localised importance of bank erosion on FWPM habitat was identified as a key factor contributing to population decline. This process though was in turn driven by greater runoff and a flashier hydrological regime linked to runoff generation on both compacted pasture and cultivated land. The results informed management of fine sediment problems relating to both diffuse water pollution and riparian corridor management, e.g., targeted management of channel banks and road crossings.

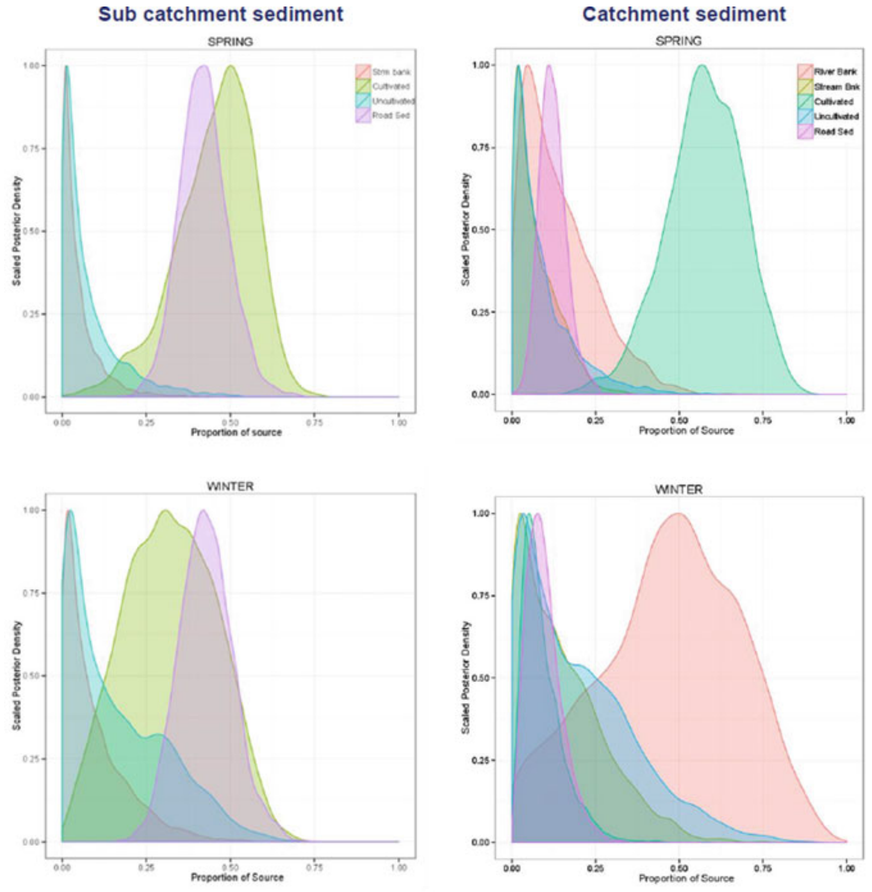

Fig. 7.8 Unmixing model outputs showing most likely source contributions at the subcatchment (left) and catchment (FWPM beds) (right) scales, in summer and winter

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Alex Taylor has been working with fallout radionuclide (FRN) tracing techniques for 15 years, with particular focus on beryllium-7 fallout and partitioning behaviour in soils and sediments. He has worked extensively on the application of FRNs for the assessment of soil erosion in the context of sustainable land management practices. As a river catchment scientist, Alex has been involved in numerous sediment source tracing studies in river systems, working with a range of governmental and non-governmental organizations to assess contaminant sources, pathways, and receptors. His work informs targeted mitigation practice within river catchments, supporting healthy soil and aquatic-marine systems.

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Chapter 8
Conclusion: Stable Isotope Tracers Are Useful for the Identification of Pollutants in Agro-ecosystems

G. Imfeld, G. Skrzypek, J. Adu-Gyamfi, and L. Heng

Pollutant management in agro-ecosystems is a complex challenge that requires two key questions to be answered: (i) how to improve identification and quantification of pollutant sources, transport pathways and fate on the catchment scale? and (ii) how to prioritise and combine soil and water management practices to minimise the use and impact of agro-pollutants? An integrative book such as this one, focusing on the stable H, C, N, O and S isotope compositions of a variety of agro-pollutants, may help to address the first question and enable increasingly precise and robust evaluation of pollution in agro-ecosystems. After understanding pollution sources and dispersal, the farm operators and land managers can attempt to answer the second question by considering local conditions and agriculture practices.

This book has highlighted that stable isotope tracers have great potential to improve the qualitative and quantitative assessment of soil and water pollution, which is crucial for ecosystem protection plans worldwide. However, stable isotope analyses should be considered as complementary to conventional concentration analysis

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and mass load calculations. Stable isotope and ion ratios are usually independent of solute concentrations and provide primarily information about pollution sources and transformation processes but not about pollution levels. Pollution levels need to be determined using hydrochemical analyses and load calculations, respectively, to volume of water and pollutant concentrations.

Various stable isotope tracers are often used individually, but the best results are achieved by adopting a multi-tracer approach to obtain more reliable characterisation of pollution sources and to tease apart the water and solute budgets. Thus, combination of the stable isotope and hydrochemical analyses of specific solutes with optimal sampling design, sediment source apportionment and stable isotope composition of water to estimate retention time and evaporation can reinforce the evaluation of sources and transport of pollutants in agro-ecosystems and help to reduce uncertainties of study outcomes. Here, we also provide examples of case studies to show how the stable isotope approaches helped to estimate sources and transport of agro-pollutants and inform decision-making on the catchment scale to control diffuse agricultural pollution and define adapted policies. The ability to enhance comprehension, identification, and quantification of sources and processes related to agro-pollutants is crucial in a global context. Indeed, chemical pollution and nutrient (nitrogen, phosphorus) flows into the biosphere and oceans have exceeded safe limits, breaching the boundaries of global sustainability, which poses a direct threat to humanity (Steffen et al. 2015).

This book also aims to present a scientific *toolbox* that gathers optimal designs of sampling programmes, considering possible mixing scenarios and multiple isotope tracers of the origin, transport and transformation for a variety of agro-pollutants (Table 8.1). The book's focus on sampling protocols, current analytical techniques, standard operating procedures, and field examples can contribute to the integration of multi-tracer approaches into routine studies and research practices. It has been demonstrated that establishing a balance between grab sampling and time-integrated sampling is essential in each study to mitigate biases when interpreting agro-pollutant sources and behavior. In the interpretation phase, providing an estimation of uncertainties related to source apportionments and process extent is advisable whenever possible. This includes error propagation to ensure a rigorous interpretation.

Most importantly, the book also addresses requirements and limitations associated with the use of each type of isotope tracer (Table 8.1). A good knowledge of stable isotope signatures of the pollution sources is an essential requirement for efficient application of all stable isotope tracers in mixing or fractionation models. In future, extensive databases for source identification and apportionment of sediment or single agro-pollutant compounds may be available, following Findable, Accessible, Interoperable, Reusable (FAIR) principles. However, agro-pollutants, whose source identification seems possible and is targeted (e.g., PO_4, NO_3), should be distinguished from agro-pollutants, whose degradation is the main process considered (e.g., pesticides). In many cases, sampling and sample handling are sensitive operations that may cause undesired isotope fractionation, as for the $\delta(^{18}O)_{PO4}$ method to investigate P cycling in the soil–plant continuum. In addition, the stable isotope composition of

Table 8.1 Current development and future prospects to evaluate agro-pollutants in the field using stable isotope techniques

	Interest in tracing agro-pollutants	Requirements	Limitations and challenges	Advantages and future developments
Conceptual design	• Establish a sampling design for tracing agricultural pollutants • Estimation of the relative contributions from pollution sources	• Hydrochemical and hydrogeological baseline data • Initial stable isotope compositions at pollution sources • Hydro-climatic records	• Requires understanding of the study area (hydrology/land use/source locations) • Isotope signatures of sources need to be statistically significantly different • Signatures of potential pollution sources to be analysed at pollution sources	• Potential use of multiple sampling points and multiple isotope tracers to identify major sources of pollution by estimating pollution budgets in different subcatchments
Mixing and fractionation models	• Achieving an optimal sampling design • increase efficiency: reducing sample number, workload and cost	• Simple mixing models can be solved algebraically • Known and significantly different stable isotope signatures of individual sources • Good mixing of water and pollution assumed	• Number of tracers used not lower than one less than the number of sources under consideration	• Advanced modelling for correction of stable isotope fractionations at the time of the reaction • Probability models for systems with multiple pollution sources
Compound-specific isotope analysis (CSIA) of pesticides	• Trace and quantify pesticide sources and degradation • Evaluate prevailing zones and periods of pesticide dissipation	• Methods to extract and concentrate pesticides from environmental samples • Chromatographic separation of all compounds • Sufficient knowledge regarding hydrological and hydroclimatic conditions and functioning	• CSIA for low pesticide concentrations in complex environmental matrices • CSIA restricted to less polar pesticides while using GC-IRMS • isotope fractionation factors to be derived in the laboratory to evaluate processes	• Pesticide enrichment and clean-up from complex matrices • Multi-element CSIA to interpret complex pollution scenarios with multiple pollution sources or different degradation processes • Comprehensive studies on the catchment scale over long periods

(continued)

Table 8.1 (continued)

	Interest in tracing agro-pollutants	Requirements	Limitations and challenges	Advantages and future developments
Stable oxygen isotope composition of phosphates	• Stable isotope composition of oxygen bond to phosphorus $\delta(^{18}O)_{PO4}$ • Investigate P cycling in the soil–plant continuum	• Extraction, isolation and purification for $\delta(^{18}O)_{PO4}$ determination from organic and inorganic P forms and fractions in soils, plants, sediments, fertilisers and water • Calculation of isotopic equilibrium and the effect of organic P hydrolysis • $\delta(^{18}O)_{H2O}$ of water required to interpret $\delta(^{18}O)_{PO4}$	• Small differences between $\delta(^{18}O)_{PO4}$ values of P pools between treatments or between different P pools • $\delta(^{18}O)_{PO4}$ strongly influenced by biotic processes. Enzymatic activity to be stopped during extraction • High quantities of organic matter or low P concentrations complicate extraction	• $\delta(^{18}O)_{PO4}$ to interpret the coupling between P, C and N cycling • Knowing $\delta(^{18}O)_{PO4}$ values of specific organic P compounds to advance earth system models • Laboratory and glasshouse studies to apply $\delta(^{18}O)_{PO4}$ in the field

(continued)

Table 8.1 (continued)

	Interest in tracing agro-pollutants	Requirements	Limitations and challenges	Advantages and future developments
Stable sulphur and oxygen isotope composition of sulphates	• Generation, transport and mixing of sulphate and stable sulphur and oxygen isotope compositions used as tracers of various sources	• Processes contributing to the stable isotope fractionation of sulphates in soil and aquatic environments should be considered • $\delta(^{2}H)_{H2O}$, $\delta(^{18}O)_{H2O}$ data support interpretation of $\delta(^{34}S)_{SO4}$ and $\delta(^{18}O)_{SO4}$ values to partition the fate of water and solutes	• Stable isotope composition of leachates, and especially $\delta(^{18}O)_{SO4}$, can be modified by bacterial processes • In catchments contaminated with pollutants from several sources, stable isotope signatures of overlapping values may be ambiguous	• Denser sampling to determine pollution budgets in individual subcatchments • Application of more advanced probabilistic models
Sediment quantification	• Source apportionment of (fine) sediment and associated co-pollutants • Sediment mobilisation, transport, storage and yield to evaluate pollutant source dynamics	• Tracing studies require large sample numbers for elemental concentrations • CSIA technique offers the possibility to discriminate different land uses	• Assumption of instantaneous ^{137}Cs, ^{210}Pb and ^{7}Be binding at the soil surface is site-dependent • Particle sorting and enrichment during sediment transport and storage should be accounted for	• Evaluating heterogeneous and complex response to management practice and erosion within the lower-order tributary catchment areas • Expanding the application and sensitivity of XRF analysis

the agro-pollutant of interest needs to be considered in relation to the other chemical compounds present in the environment, which may contribute to stable isotope exchange, equilibration or chemical synthesis, thus altering the original signatures of the pollution source. In situations where degradation or transformation is anticipated, understanding the associated isotope fractionation is crucial for interpreting field data. If this information is not yet available in the literature, isotope fractionation may be deduced from reference experiments (e.g., biodegradation in native sediment, photolysis in river water). Moreover, separation fractionation from mixing is the primary challenge and may sometimes lead to ambiguous results. Finally, the interpretation of agro-pollutant transformation is constrained by the absence of reference field studies, encompassing vegetation and ecosystem dynamics. Therefore, laboratory microcosms and glasshouse studies will be instrumental in advancing the application of stable isotope tracers in agro-ecosystems. Additionally, they will contribute to the development of novel, mechanistic mixing concepts and models to enhance studies in complex and dynamic agro-ecosystems.

Despite the acknowledged limitations, stable isotope techniques provide additional dimensions to the interpretation of agro-pollutants, enabling advanced studies that are not achievable through traditional hydrochemical methods alone. This book contributes to the systematization, standardization, and optimization of stable isotope methods in routine studies of agro-pollutants on a catchment scale. We anticipate that, in the future, stable isotope methods will steadily become the routine and preferred approach in many research areas, including the tracing of agro-pollutants.

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G. Skrzypek has 24 years of experience in the field of stable isotope geochemistry, hydrochemistry, and ecology since his PhD (1999) from The University of Wrocław (UWr). Grzegorz is currently working as Associate Professor and Coordinator of MSc Biology and Ecology specializations at The University of Western Australia (UWA). Grzegorz has published many papers on applications of stable isotope techniques in hydrological, hydrochemical, pollution, ecological, and paleoclimate studies. He uses stable H, C, N, O, S, and Sr isotopes to trace water budget, nutrient fluxes in natural environments, and dispersal of pollution in environments impacted by mining and agriculture. He held a prestigious Australian Research Council Future Fellowship at UWA (2012–2015) and Foundation for Polish Science Scholarship at UWr (1999–2000). Currently, he is serving as the deputy director of the West Australian Biogeochemistry Centre at UWA (wabc.uwa.edu.au). Grzegorz has been a chief investigator on a number of research projects in Europe and Australia and an expert on IAEA Technical Cooperation Projects and IAEA Technical Meetings.

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Glossary

^{31}P-NMR ^{31}P nuclear magnetic resonance. Spectroscopic technique, based on the spins of atomic nuclei, which is used to study mainly organic P in soils and plants.

Adsorption Bonding of ions, molecules, or atoms in the soil solution or soil air to sorption sites of solids through physical and chemical processes.

ARD Acid Rock Drainage, sulfide oxidation process leading to generation of sulfates and acidification of water.

Biopores Pores that formed through the activity of roots and/or soil macro-fauna like earthworms.

CSIA Compound-specific isotope analysis. CSIA refers to the measurement of the isotope ratios (typically carbon, hydrogen, oxygen, nitrogen, sulfur, or chlorine) of individual organic compounds extracted from complex environmental mixtures.

Desorption Release of ions, molecules, or atoms sorbed to solids into the soil solution or air. Reverse process to adsorption.

DGT Diffusive gradient in thin film technique. Used as a measure for plant-available phosphorus.

Dissolution It refers to the disintegration of solid minerals into individual ions/complexes and released into the soil solution. Opposite process of precipitation. Influenced by factors like soil pH and concentration.

EU15 Number of member countries of the European Union prior to its enlargement on May 1, 2004. It includes the following countries: Austria, Belgium, Denmark, Finland, France, Germany, Greece, Ireland, Italy, Luxembourg, Netherlands, Portugal, Spain, Sweden, and UK.

Genomics Analysis of the complete set of DNA (including its genes) of an organism.

Immobilization Conversion of an element, compound, or ion into an unavailable form. In the case of P, it often refers to the uptake of P by microorganisms.

Isotopologue Molecules such as phosphate and phytate which differ in their isotopic composition are called isotopologues. For example, a phytate containing only ^{18}O and a phytate molecule containing only ^{16}O are two isotopologues of the same molecule.

Metabolomics Investigating cellular processes involving metabolites like nucleotides.

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Metagenomics Extraction of the genome directly from environmental samples and analysis of the genome without prior cultivation of microorganisms.

Mineralization Conversion of an element, compound, or ion from an unavailable form into a bioavailable form.

Mycorrhizal fungi Symbiosis between certain soil fungi and the roots of higher plants.

NanoSIMS Nano-scale secondary ion mass spectrometry. Spectrometric method with a high resolution which allows, for example, the measurement of different isotopes on a cellular and sub-cellular level.

Omics Umbrella term for research areas ending with -omics like metagenomics and proteomics.

Phosphorus use efficiency The ability of a plant to grow well under P limitation. In agriculture, it relates to the ability of a crop to acquire P and produce a relatively high yield also under P limitation. It is often calculated based on the total amount of P in a plant and the amount of applied fertilizer P.

Porosity It refers to the volume of pores in soils or sediments, expressed in percent. The pores include air and water-filled pores.

Precipitation of minerals It refers to the precipitation of dissolved ions from the soil solution when, for example, the soil pH or the concentration changes. Opposite process of dissolution.

Proteomics Analysis of all proteins within a cell/organism at a certain time. It is often used to investigate what proteins are expressed when organisms are exposed to stress, such as phosphorus limitation.

PSR Phosphate starvation response of organisms. Includes expression of certain genes under P starvation and changes in root architecture.

Rhizosphere The soil volume which is affected by root activity. It typically relates to the first few mm around roots.

Rock phosphate The main raw material for mineral phosphorus fertilizer.

Sorption General term for processes that lead to the attachment of one substance to another. Includes adsorption, absorption, and ion exchange. Reverse process to desorption.

SPE Solid phase extraction.

Transcriptomics Investigating the transcriptome (sum of RNA transcripts) of an organism.

XRF X-ray fluorescence analytical technique used for identification of minerals.