Chapter 11
Total Phosphorus, Nitrogen and Carbon in Leaf Litter

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Keywords Acid digestion · Alkaline digestion · Elemental analyser · Kjeldahl · Leaf conditioning · Litter quality · Loss on ignition · Nutrient immobilization · Spectrophotometry · Wet chemical analysis

1 Introduction

Plant litter decomposition is a fundamental ecosystem process in forests that sustains soil food webs (Gessner et al. 2010; Garcia-Palacios et al. 2016). In many freshwater, coastal and marine environments, plant litter of various origins is also abundant and an important source of energy and nutrients for aquatic food webs (e.g. Wolanski and Elliott 2015; Garcia-Palacios et al. 2016). Nitrogen (N) and phosphorus (P) are important nutrients determining the quality and decomposability of plant litter (Enriquez et al. 1993), although other litter characteristics also play important roles in controlling rates of litter decomposition (Webster and Benfield 1986; Ostrofsky 1997; Aerts 1997; Abelho 2001). Most P is used by biota for the

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Table 11.1 Summary of nitrogen and phosphorus concentrations as well as C:N and C:P molar ratios in plant litter of a broad range of species. Except for macroalgae, plant litter refers to leaves

Plant material	N (%)	C:N	P (%)	C:P	Reference
2 macroalgae	2.50–3.92	12.6–18.7	0.19–0.36	224–544	1
2 seagrass species	2.64–2.76	15.6–24.3	0.50–0.55	191–258	1
4 freshwater angiosperms	1.37–3.44	12.7–27.8	0.43–0.85	140–249	1
6 *Carex* spp.	0.18–1.07	46.1–315	0.016–0.150	729–7847	1
2 *Pinus* spp.	0.40–1.51		0.017–0.131		1
5 *Acer* spp.	0.73–1.71	24.1–60.9	0.112–0.411		2
2 *Salix* spp.	0.83–2.24	21.0–56.5	0.121–0.281		2
3 *Populus* spp.	0.92–2.38	19.7–52.4	0.083–0.092		2
Alnus glutinosa	1.38–2.98		0.02–0.15		3
Quercus robur	0.56–2.13		0.01–0.35		3
Fraxinus excelsior	1.1		0.073		4
Fagus sylvatica	0.68		0.032		4
Prunus avium	0.52		0.048		4
Corylus avellana	1.05		0.072		4
Platanus hybrida	0.52		0.017		4
67 temperate riparian tree species	0.16–3.20		0.010–0.300		5
101 tropical riparian tree species	0.18–3.84		0.010–0.230		5

1 = Enriquez et al. (1993); 2 = Ostrofsky (1997); 3 = Graça and Poquet (2014); 4 = Gessner and Chauvet (1994); 5 = Boyero et al. (2017); 6 = Pérez-Harguindeguy et al. (2000)

synthesis of RNA, DNA, ATP and phospholipids, whereas N is mainly associated with chitin and proteins but also an important component of nucleic acids and some secondary plant metabolites (Sterner and Elser 2002). Litter characterized by low ratios of C:N and C:P (i.e. high N and P concentrations) and low concentrations of structural plant polymers such as lignin tend to decompose fast, whereas litter containing nutrients at low concentrations tend to be more recalcitrant (Flindt et al. 1999; Kadlec and Wallace 2009). Typical concentrations of N and P in plant litter from diverse environments are summarized in Table 11.1, based on a comprehensive compilation of nutrient concentrations in more than 250 photosynthetic organisms ranging from phytoplankton to trees (Enríquez et al. 1993), 48 deciduous tree leaf species (Ostrofsky 1997), riparian leaf litter analysed in a global-scale study (Boyero et al. 2017) and a meta-analysis of C:N and C:P ratios of leaf litter in aquatic environments from 44 primary studies (Kennedy and El-Sabaawi 2017).

A large fraction of the P is rapidly leached from dead leaf tissue during the initial phase of decomposition, especially when autumn-shed leaves dry before they enter the aquatic environment (Gessner 1991; Flindt et al. 1999). N is not generally leached upon senescence and death, although initially decreasing concentrations have occasionally been observed (Meyer and Johnson 1983). Subsequently, N and P concentrations of leaves usually increase during decomposition (Webster and Benfield 1986; Gessner 1991). This increase is attributed to microbial colonization (Gulis and Suberkropp 2003; Gulis et al. 2006; Ferreira et al. 2006, 2015), which

enhances the nutritional quality of leaf litter for detritivores (Webster and Benfield 1986; Bärlocher and Sridhar 2014).

Total N in litter can be determined by the standard Kjeldahl method for plant samples or a modification thereof (Ferskvandsbiologisk Laboratorium University of Copenhagen 1992a). Leaf-litter samples are dried, homogenized and digested in concentrated sulphuric acid to reduce all nitrogen species to ammonium (NH_4^+). After neutralizing the resulting solution, it is filtered and analysed spectrophotometrically. Alternatively, the total N concentration of litter can be quantified by means of a CHN elemental analyser. Advantages are that the analysis is accurate and easy and that total carbon is determined simultaneously, enabling the calculation of C:N ratios based on analyses of both elements in the same sample. However, the equipment is costly and not readily available in all laboratories.

Total P can also be analysed spectrophotometrically after digestion. Dried and homogenized litter samples are digested by autoclaving with sodium hydroxide and sodium persulphate or the samples are first combusted and the ash digested in concentrated hydrochloric acid (Ferskvandsbiologisk Laboratorium University of Copenhagen 1992b). The resulting solutions are filtered and total P is quantified as orthophosphate (PO_4^{3-}) by using the ascorbic acid method. Both procedures are presented here. The first method is preferable when individual plant samples are large. The second method is advantageous when many samples are to be processed or the amount of plant material is low, because the method is fast and handling of small amounts of ash is avoided.

A combined method for total N and total P determination can be performed by the total N procedure. However, the pH during neutralization of samples must never exceed 6, otherwise dissolved phosphorus will precipitate. The samples are then filtered and analysed spectrophotometrically. As N and P are rapidly recycled by biological processes, samples should in all cases be analysed as soon as possible after the digestion step. When this is not possible, samples should be preserved by acidification, refrigeration, or freezing, depending on the method.

2 Phosphorus Using an Acid Digestion Method

2.1 Equipment and Material (Acid-Washed Glassware)

- Drying oven (50 °C)
- Mill or mortar and pestle
- Spectrophotometer
- Cuvettes
- Hot plate
- pH meter
- Analytical balance (± 0.1 mg)
- Muffle furnace

- Porcelain crucibles
- Desiccator
- Vortex
- Glass fibre filters (Whatman GF/C or equivalent)
- Syringes (20 ml) with adapter for $\varnothing$ 25 mm glass fibre filters
- Pipettes (200, 1000, 5000 and 10,000 μl)
- Erlenmeyer flasks (50–100 ml)
- Glass tubes (> 15 ml)
- Cooling bulbs
- Gloves

2.2   Chemicals (Analytical Grade)

- Deionized water
- HCl (37%) – concentrated hydrochloric acid
- KH_2PO_4 – potassium dihydrogen phosphate
- 2-$[(CH_3)_2CH]C_6H_3$–5-$(CH_3)OH$ – thymol (crystalline)
- $(NH_4)_6Mo_7O_{24} \bullet 4\ H_2O$ – ammonium heptamolybdate tetrahydrate
- H_2SO_4 (98%) – sulphuric acid concentrate
- $K(SbO)C_4H_4O_6 \bullet 5\ H_2O$ – potassium antimony (III) oxide tartrate pentahydrate, extra pure
- $C_6H_8O_6$ – L(+)ascorbic acid (vitamin C)

2.3   Solutions

- Solution 1 – PO_4^{3-} stock solution (40 mg P l^{-1}): dissolve 175.75 mg KH_2PO_4 in 1000 ml of deionized water, add one crystal of thymol, and store the stock solution for up to 3 months at ambient temperature.
- Solution 2 – PO_4^{3-} working solution (10 mg P l^{-1}) for preparing a standard curve: dilute 4 ml of the stock solution in 1000 ml of deionized water; this working solution must be freshly prepared every day.
- Solution 3 – stock solution of reagent; to 12 g of $(NH_4)_6Mo_7O_{24} \bullet 4\ H_2O$ in 500 ml of deionized water, add very carefully 140 ml of concentrated H_2SO_4. After mixing and cooling, add 275 mg of $K(SbO)C_4H_4O_6 \bullet 5\ H_2O$ and let it dissolve, then adjust the volume to 1000 ml with deionized water, and store the solution for up to 3 months at ambient temperature.
- Solution 4 – working solution of reagent: immediately before use add 1.06 g of ascorbic acid to 100 ml of the stock reagent solution.

2.4   Sample Preparation

1. Dry and grind plant material, and then put it into porcelain crucibles.
2. Place the samples in a muffle furnace for 4 hours at 500 °C.
3. Determine dry and ash-free dry mass of the samples as indicated in the C determination method (see Sect. 6.2).
4. Weigh portions of approximately 5 mg ash to the nearest 0.1 mg.
5. Place the sample in a 50 or 100 ml Erlenmeyer flask with 25 ml of deionized water.
6. Add 1 ml of concentrated HCl.
7. Place the Erlenmeyer flask on a heating plate to evaporate the water.
8. Place a cooling bulb on top of the flask and wait for a few minutes until the solution starts tingling gently.
9. Continue heating the samples (temperature < 110 °C, because the boiling point of HCl is 110 °C) until the solution turns yellow and translucid.
10. Rotate the flask from time to time to remove ash particles from the walls (wear gloves!).
11. If the water evaporates before the solution turns yellow and translucid, add more HCl in 1 ml aliquots.
12. Transfer the solution to a 100 ml flask and adjust the sample volume to 100 ml with deionized water.
13. Filter the samples through a glass fibre filter connected to a 20 ml syringe.
14. Analyse the filtered samples immediately after extraction or, if not possible, store them frozen at −20 °C.

2.5   Spectrophotometric Analysis

1. Run a standard curve using concentrations of 40, 80, 100, 200, 400 and 800 µg P l^{-1} (Table 11.2).
2. Dilute samples if higher P concentrations are expected, because the standard curve is linear only up to 1000 µg P l^{-1}.
3. Transfer 10 ml of sample into a glass tube.

Table 11.2   Preparation of the standard curve to calculate PO_4^{3-} concentrations

PO_4^{3-} working solution (ml)	Deionized H_2O (ml)	Final volume (ml)	Final P concentration(µg l^{-1})
0.8	99.2	100	80
1.0	99.0	100	100
2.0	98.0	100	200
4.0	96.0	100	400
8.0	92.0	100	800

4. Add 1 ml of the working reagent and vortex.
5. Wait for 15 min for the reaction to complete
6. Measure absorbance at 882 nm, using deionized water as reference.
7. Use as a blank 10 ml of deionized water treated like the samples.
8. Calculate the P concentration of the samples based on the standard curve, taking into account the absorbance reading, blank values and moisture and ash contents of the litter samples.

3 Phosphorus Using an Alkaline Digestion Method

3.1 Equipment and Material (Acid-Washed Glassware)

- Drying oven
- Mill or mortar and pestle
- Porcelain crucibles
- Desiccator
- Analytical balance (±0.1 mg)
- Spatula
- Pipettes (1000, 5000 and 10,000 μl)
- Extraction glass tubes with cap (> 30 ml; e.g. Pyrex tubes)
- Autoclave
- Fridge
- Syringes (20 ml) with adapter for ⌀ 25 mm glass fibre filters
- Syringe filters (Whatman GF/C or equivalent)
- Glass tubes (> 15 ml)
- pH paper
- Spectrophotometer
- Cuvettes

3.2 Chemicals

- Ethanol
- Deionized water
- NaOH – sodium hydroxide
- $Na_2S_2O_8$ – sodium persulphate
- H_2SO_4 (96–98%) – concentrated sulphuric acid
- $K(SbO)C_4H_4O_6 \bullet 0.5H_2O$ – potassium antimonyl tartrate hemihydrate
- $(NH_4)_6Mo_7O_{24} \bullet 4H_2O$ – ammonium molybdate tetrahydrate
- $C_6H_8O_6$ – ascorbic acid (vitamin C)
- KH_2PO_4 – potassium dihydrogen phosphate

3.3   Solutions

- Solution 1–1 M sodium hydroxide: dissolve 3.9997 g NaOH in 100 ml of deionized water, and keep indefinitely in the dark at room temperature.
- Solution 2–3 M sodium hydroxide: dissolve 12 g NaOH in 100 ml of deionized water, and keep indefinitely in the dark at room temperature.
- Solution 3–0.525 M sodium persulphate: dissolve 12.5 $Na_2S_2O_8$ g in 100 ml of deionized water, and keep at 4 °C for up to several months.
- Solution 4–15% sulphuric acid: add 15 ml concentrated H_2SO_4 to 75 ml of deionized water, and keep at 4 °C for up to 2 months.
- Solution 5 – potassium antimonyl tartrate (2.8 g l^{-1}): dissolve 0.14 g K(SbO) $C_4H_4O_6 \bullet 0.5\ H_2O$ in 50 ml of deionized water, and keep at 4 °C for up to 1 month.
- Solution 6 – ammonium molybdate (40 g l^{-1}): dissolve 2 g $(NH_4)_6Mo_7O_{24} \bullet 4H_2O$ in 50 ml of deionized water, and keep at 4 °C in the dark for up to 2–3 weeks.
- Solution 7 – combined reagent: mix 50 ml of 15% sulphuric acid +5 ml of potassium antimonyl tartrate +15 ml of ammonium molybdate +30 ml of deionized water, and keep at 4 °C in the dark for up to 2–3 weeks.
- Solution 8 – ascorbic acid (20 g l^{-1}): always freshly prepare solution by dissolving 1 g vitamin C in 50 ml of deionized water.
- Solution 9 – concentrated potassium dihydrogen phosphate (50 mg P l^{-1}): dissolve 21.97 mg KH_2PO_4 in 100 ml of deionized water, and keep at 4 °C for up to 2 months.
- Solution 10 – diluted potassium dihydrogen phosphate (1 mg P l^{-1}): mix 1 ml of the concentrated KH_2PO_4 with deionized water, and make up to 50 ml.
- Solution 11 – diluted KH_2PO_4, (2 mg P l^{-1}): if high P concentrations are expected in the samples, mix 2 ml of concentrated KH_2PO_4 with deionized water, and make up to 50 ml.

3.4   Sample Preparation

1. Grind samples and determine dry mass and ash-free dry mass as indicated in Sect. 6.2.
2. Weigh 2–4 mg (± 0.1 mg) of ground plant material and transfer to labelled glass tubes.
3. Add 1 ml of 1 M NaOH, which results in a bright yellow translucid coloration.
4. Add 2.38 ml of 0.525 M sodium persulphate.
5. Add 6.62 ml of deionized water and make up to a total volume of 10 ml.
6. Loosely close the tubes and place them inside the autoclave, noting the tube positions in the racks and in the autoclave as the labelling may be erased.
7. Autoclave at 120 °C for 2 h.

8. Let the samples cool until the solution's yellow coloration disappears and the litter turns grey or whitish.
9. Check the pH with pH paper to ensure it is between 2 and 7
10. If outside this range, adjust the pH by adding 15% H_2SO_4 or 3 M NaOH (e.g. 4 mg alder litter results in a pH of ~1.5, which can be raised to 3 by adding 0.5 ml of NaOH).
11. Adjust the volume in the tube to 25 ml with deionized water.
12. Filter 10 ml of the sample into a glass tube.

3.5 Spectrophotometric Analysis

1. Add consecutively the volumes indicated in Table 11.3 of Solution 10 (KH_2PO_4), Solution 1 (1 M NaOH), Solution 3 (0.525 M $Na_2S_2O_8$) and deionized water, including the last two concentrations in the table only if high P concentrations are expected.
2. Dilute samples when the final P concentrations are high, because the standard curve is linear only up to 1000 µg P l^{-1} (Table 11.3).
3. Add 2 ml of combined reagent (Solution 7) to the tubes, including to those used to establish the standard curve.
4. Add 0.5 ml of ascorbic acid solution.
5. Wait for 30 min for the reaction to complete.
6. Determine absorbance in the spectrophotometer at 880 nm, using deionized water as reference.
7. Use as a blank 10 ml of deionized water treated like a sample.

Table 11.3 Preparation of solutions to establish a standard curve for the determination of P concentrations in leaf litter

KH_2PO_4 solution (mg l^{-1})	KH_2PO_4 solution (ml)	1 M NaOH (ml)	0.525 M $Na_2S_2O_8$ (ml)	Deionized H_2O (ml)	Final volume (ml)	Final P concentration (µg l^{-1})
1	0	1	2.38	6.62	10	0
1	0.05	1	2.38	6.57	10	5
1	0.10	1	2.38	6.52	10	10
1	0.25	1	2.38	6.37	10	25
1	1.5	1	2.38	5.12	10	150
1	2.5	1	2.38	4.12	10	250
1	4.0	1	2.38	2.62	10	400
1	5.0	1	2.38	1.62	10	500
2	5.0	1	2.38	1.62	10	1000

8. Calculate the P concentration of the samples based on the standard curve, taking into account the absorbance reading, blank values and moisture and ash contents of the litter samples.

4   Nitrogen by a Modified Kjeldahl Method

4.1   Equipment and Material

- Drying oven (50 °C)
- Mill or mortar and pestle
- Spectrophotometer
- Hot plate
- pH meter
- Analytical balance (± 0.1 mg)
- Porcelain crucibles
- Desiccator
- Vortex
- Glass fibre filters (Whatman GF/C or equivalent)
- Syringes (20 ml) with adapter for Ø 25 mm glass fibre filters
- Pipettes (200, 1000 and 5000 μl)
- Erlenmeyer flasks (50–100 ml)
- Glass tubes (> 15 ml)
- Cooling bulbs
- Gloves

4.2   Chemicals (Analytical Grade)

- Deionized water
- $CuSO_4 \bullet 5\ H_2O$ – copper sulphate pentahydrate
- H_2SO_4 (96–98%) – concentrated sulphuric acid
- $(NH_4)_2SO_4$ – ammonium sulphate
- $2\text{-}[(CH_3)_2CH]C_6H_3\text{–}5\text{-}(CH_3)OH$ – thymol (crystalline)
- C_6H_5OH – phenol (crystalline)
- $Na_2[Fe(CN)_5NO] \bullet 2\ H_2O$ – sodium nitroprusside dihydrate
- NaOH – sodium hydroxide
- NaOCl (15%) – 15% sodium hypochlorite solution

4.3   Solutions

- Solution 1 – copper sulphate: dissolve 10 g $CuSO_4 \cdot 5\ H_2O$ in 100 ml of deionized water.
- Solution 2 – ammonium stock solution (100 mg N l^{-1}): dissolve 0.472 g $(NH_4)_2SO_4$ in 1000 ml of deionized water, add one crystal of thymol, and keep the stock solution for up to 3 months at ambient temperature.
- Solution 3 – ammonium working solution (10 mg N l^{-1}) for preparing a standard curve: dilute 10 ml of the stock solution in 1000 ml of deionized water; this working solution must be freshly prepared every day.
- Solution 4 – reagent A: dissolve 50 g phenol and 0.25 g sodium nitroprusside dihydrate in 1000 ml of deionized water.
- Solution 5 – reagent B: dissolve 25 g NaOH and 20 ml 15% NaOCl in 1000 ml of deionized water.

4.4   Sample Preparation

1. Determine fresh and dry mass of litter samples (see Sect. 6.2).
2. Weigh portions of dried and ground litter samples ($\leq$2 mg) to the nearest 0.1 mg.
3. Place the sample in an Erlenmeyer flask with 25 ml of deionized water.
4. Add 0.2 ml of Solution 1 and 1 ml of H_2SO_4.
5. Place the Erlenmeyer flasks on a hot plate and boil off the water (wear gloves!).
6. When the water has evaporated and a light smoke appears, place a cooling bulb on top of the flasks, which will result in a gentle tingling after a few minutes.
7. Observe the start of the organic matter digestion (temperature ~150 °C; the boiling point of H_2SO_4 is 290 °C), which is noticeable by the appearance of a dark coloured tar that is subsequently mineralized to CO_2 and H_2O, as indicated by white smoke appearing inside the flask.
8. Rotate the flask from time to time to remove residues from the walls.
9. If necessary, add more H_2SO_4 in 1 ml aliquots until the digestion is complete, as indicated by the acid solution becoming light yellow and translucid.
10. Continue the digestion for another 30 min.
11. After cooling, transfer the solution to a beaker and rinse the flask walls with deionized water, ignoring that the solution temperature will exceed 30 °C.
12. Adjust the samples with 1 M NaOH to the point where $Cu(OH)_2$ flocculates (pH ~3), ignoring that the solution temperature may increase to >50 °C.
13. Use a dilute (0.1 M) NaOH solution to raise the pH to 5–8, being most careful because pH shifts rapidly from 3 to 10 and ensuring that it is similar among all samples.
14. Adjust the sample volume to 100 ml with deionized water.
15. Filter the samples through a glass fibre filter connected to a 20 ml syringe.

Table 11.4 Preparation of the standard curve to calculate NH_4^+ concentrations

NH_4^+ working solution (10 mg N l^{-1})	Deionized H$_2$O (ml)	Final volume (ml)	Final N concentration (µg l^{-1})
0.5 ml	99.5	100	50
1.0 ml	99	100	100
2.0 ml	98	100	200
4.0 ml	96	100	400
8.0 ml	92	100	800

16. Analyse samples immediately or, if not feasible, store acid samples at 4 °C for up to 2 months or neutralized and filtered samples at −20 °C for up to 6 months.

4.5 Spectrophotometric Analysis

1. Run a standard curve with concentrations of 50, 100, 200, 400 and 800 µg N l^{-1} as shown in Table 11.4.
2. Dilute samples if higher N concentrations are expected, because the standard curve is linear only up to 1000 µg N l^{-1}.
3. Transfer 10 ml of sample into a glass tube.
4. Add 1 ml of reagent A and vortex.
5. Wait for about 1 min, and then add 1 ml of reagent B and mix again; if many samples are processed simultaneously, pipetting can be staggered such that the delay between the addition of reagents A and B is about 1 min.
6. Protect the developing blue colour from sunlight.
7. Wait for a defined time, but at least 1 h for the reaction to complete.
8. Measure absorbance at 630 nm, using deionized water as reference.
9. Use as a blank 10 ml of deionized water treated like the samples.
10. Calculate the N concentration of the sample based on the standard curve, taking into account the absorbance reading, blank values and moisture content of the litter sample.

5 Total Nitrogen and Carbon with an Elemental Analyser

5.1 Equipment and Material

- Drying oven (50 °C)
- Desiccator
- Analytical balance (± 0.1 mg)
- Mill or mortar and pestle
- CHN Elemental Analyser
- Metal cups (tin, aluminium or silver) suitable for the CHN analyser

5.2   Procedure

1. Calibrate the CHN analyser according to the instructions of the manufacturer.
2. Grind the dry plant material in a mill or with mortar and pestle (powder <1 mm particle diameter), and carefully clean the equipment between grinding successive samples.
3. Weigh 2–5 mg dry mass of ground litter in suitable metal cups for the CHN analyser.
4. Determine the C and N contents of the litter samples and appropriate standards according to the instructions of the manufacturer.
5. Calculate the N and C contents of the litter samples by taking into account the measured values and the moisture and ash contents of the samples.
6. Calculate the molar or atomic C:N ratio of the litter samples by taking into account the measured C and N contents as well as the atomic masses of C and N.

6   Total Carbon by a Combustion Method

6.1   Equipment and Material

- Drying oven (50 °C)
- Mill or mortar and pestle
- Analytical balance (± 0.1 mg)
- Muffle furnace (500 °C)
- Porcelain crucibles
- Desiccator

6.2   Procedure

1. Place pre-weighed fresh plant material in the oven and dry to constant weight following a standard protocol (e.g. 50 °C for at least 48 h).
2. Grind the dry plant material in a mill or with mortar and pestle (powder <1 mm particle diameter); carefully clean the equipment between grinding successive samples.
3. Place the ground litter in pre-weighed porcelain crucibles and dry at 50 °C for 48 h.
4. Cool the samples to room temperature in a desiccator and re-weigh to calculate dry mass.
5. Ignite the samples in the muffle furnace for at least 3 h at 500 °C.
6. Cool the ashes to room temperature in a desiccator and weigh.
7. Calculate litter ash-free dry mass.
8. Estimate carbon content by assuming 50% in litter AFDM, which is a reasonably good approximation for a wide variety of leaf litter types.

7   Final Remarks

Leaf litter samples collected in the field must be handled carefully to minimize external or between-sample contamination. Always use very clean material and latex gloves. Gently clean litter samples to remove as much sediment as possible, without scraping off the microbial biofilm. Use stream water for this purpose that has been passed through a 100 μm mesh screen.

Results can be expressed as %N and %P of leaf litter dry mass, if samples are not contaminated by mineral particles. Otherwise, it is preferable to measure the ash content in a representative subsample and express the data in terms of %N or %P ash-free dry mass. This can be particularly important for samples from calcareous streams where travertine precipitates can incrust decomposing litter (Martínez et al. 2015). Alternatively, nutrient content in litter can be expressed as elemental ratios (C:N, C:P and N:P), as is common in analyses of ecological stoichiometry (Sterner and Elser 2002). The molar or atomic ratio represents the proportion between elements in the number of atoms; therefore, the molar mass of each element must be considered in the calculations. For example, a sample containing 50% C and 2.92% N, the ratio is calculated as follows: molar C:N = (50/12)/(2.92/14), where 12 and 14 refer to the atomic mass of C and N, respectively.

When assessing litter nutrient dynamics during the decomposition process, it is often essential to ensure an initially high temporal resolution to capture the leaching losses, particularly of P, within the first 24–48 h of litter submergence in streams or other aquatic environments (e.g. Gessner 1991).

Ebina et al. (1983) have devised an alternative method to determine total N and P concentrations simultaneously in a single procedure. This approach greatly reduces the total analysis time. The combined analysis is facilitated by inducing a pH shift during the sample digestion, resulting in a sequential digestion of N and P species. A downside is that the method is less robust than the standard methods described in this chapter, requiring careful analyses by skilled analysts and care not to exceed the total N contents in the analysed samples (100 μg N with the described procedure). However, the method has been successfully applied to leaf litter (e.g. Gessner et al. 1998).

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