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Macrophyte communities in unimpacted European streams: variability in assemblage patterns, abundance and diversity

Annette Baattrup-Pedersen[1,*], Krzysztof Szoszkiewicz[2], Rebi Nijboer[3], Mattie O'Hare[4] & Teresa Ferreira[5]

[1]*Department of Freshwater Ecology, National Environmental Research Institute, Vejlsøvej 25, P.O. Box 314, DK-8600 Silkeborg, Denmark*
[2]*Department of Ecology and Environmental Protection, Agricultural University of August Cieszkowski, ul. Piątkowska 94C, 61-691 Poznan, Poland*
[3]*Green World Research, Alterra, P.O. Box 47, 6700 AA Wageningen, The Netherlands*
[4]*Winfrith Technology Centre, Centre for Ecology and Hydrology, DT2, 8ZD Dorchester, Dorset, UK*
[5]*Forestry Department, Agronomy Institute, Technical University of Lisbon, Tapada da Ajuda, 1349-017 Lisboa, Portugal*
*(*Author for correspondence: E-mail: abp@dmu.dk)*

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Abstract

Macrophytes are an important component of aquatic ecosystems and are used widely within the Water Framework Directive (WFD) to establish ecological quality. In the present paper we investigated macrophyte community structure, i.e., composition, richness and diversity measures in 60 unimpacted stream and river sites throughout Europe. The objectives were to describe assemblage patterns in different types of streams and to assess the variability in various structural and ecological metrics within these types to provide a basis for an evaluation of their suitability in ecological quality assessment. Macrophyte assemblage patterns varied considerably among the main stream types. Moving from small-sized, shallow mountain streams to medium-sized, lowland streams there was a clear transition in species richness, diversity and community structure. There was especially a shift from a predominance of species-poor mosses and communities dominated by liverwort in the small-sized, shallow mountain streams to more species-rich communities dominated by vascular plants in the medium-sized, lowland streams. The macrophyte communities responded to most of the features underlying the typological framework defined in WFD. The present interpretation of the WFD typology may not, however, be adequate for an evaluation of stream quality based on macrophytes. First and most important, by using this typology we may overlook an important community type, which is characteristic of small-sized, relatively steep-gradient streams that are an intermediate type between the small-sized, shallow mountain streams and the medium-sized, lowland streams. Second, the variability in most of the calculated metrics was slightly higher when using the pre-defined typology. The consistency of these results should be investigated by analysing a larger number of sites. Particularly the need of re-defining the typology to improve the ability to detect impacts on streams and rivers from macrophyte assemblage patterns should be investigated.

Introduction

Historically, vegetation has changed in streams and rivers in all Europe. Before the vast tree clearances in the Neolithic, the vegetation must have been much sparser than now because of more intensive shading from the riparian area. Even at that time, however, macrophyte communities may have been an important biological characteristic of many streams and rivers. Thus, recent studies (Svenning, 2002 and references herein) have documented that open vegetation was widespread in river floodplains throughout north-western Europe in past oceanic interglacials and the pre-agricultural Holocene, i.e., before the onset of strong human impact. Consequently, the conditions may have been suitable for macrophyte growth in many stream and river reaches, and variable physical conditions and good water quality may have supported rich vegetation.

While paleoecological evidence adds to our knowledge of past conditions in floodplains, we are entirely dependent on published records of stream vegetation to improve our understanding of assemblage patterns before the onset of strong human impact. The first published records of macrophyte surveys in Europe are from the late 1800s (Baggøe & Ravn, 1896; Raunkiær, 1895–1999; Mountford, 1994; work cited in Preston, 1995). These records give an indication of very rich and abundant vegetation. Many slow-growing species, such as broad-leaved Potamogeton species (*P. lucens* L., *P. natans* L., *P. polygonifolius* Pourret, *P. praelongus* Wulf., *P. alpinus* Balbis), for example, were very common at that time in many European lowland streams and rivers (Riis & Sand-Jensen, 2001 and references herein). During recent decades the vegetation has undergone pronounced changes. Physical degradation of the stream channel (channelisation, regulation for hydropower and navigation purposes, weed cutting and dredging) and eutrophication have resulted in a loss of many particularly slow-growing species, whereas many fast-growing species with a high dispersal capacity have increased in abundance (Carbiener et al., 1990; Mesters, 1995; Riis & Sand-Jensen, 2001).

Despite our knowledge of the adverse effects of various human activities on the vegetation in streams and rivers, no investigations have deliberately distinguished between unimpacted, slightly or highly impacted stream and river sites in previous macrophyte classifications (e.g., Butcher, 1933; Holmes et al., 1998; Riis et al., 2000). Therefore, the existing knowledge on macrophyte assemblage patterns in unimpacted European streams and rivers is limited, particularly regarding stream and river types that are situated in highly impacted areas (particularly lowland sites). In the present paper we will characterise the macrophyte communities in different types of unimpacted streams and rivers in Europe. We will use the stream typology defined in a previous EU project (AQEM) (http://www.aqem.de), which is based on ecoregion (according to Illies, 1978), size class (based on catchment area), geology of the catchment, and altitude class (Hering et al., 2004) and extended in the STAR project (Hering & Strackbein, 2001). This typology has proven useful for an assessment system based on macroinvertebrates (Verdonschot & Nijboer, 2004), but no attempts have been made to evaluate this typology for the macrophyte communities. The first objective of this study was to describe community assemblage patterns and their variation within and among these *a priori* defined stream types and to evaluate the typology by characterising assemblage patterns independently using ordination techniques. The second objective was to assess the natural variability in macrophyte-based metrics also to provide a basis for an evaluation of their suitability in ecological quality assessment.

Methods

Site selection

A total of 288 stream sites were selected in the STAR project. These sites were classified using the stream typology defined in a previous EU project (AQEM) (http://www.aqem.de), which is based on ecoregion (according to Illies, 1978), size class (based on catchment area), geology of the catchment, and altitude class (Hering et al., 2004), and extended in the STAR project (Hering & Strackbein, 2001). The sites covered an impact gradient from sites of high ecological quality (*sensu* WFD) to sites of poor or bad ecological quality (*sensu* WFD). Sites were chosen so that only one major impact was allocated to each site being either organic pollution, toxic pollution or habitat degradation. For the purpose of our study, we only included unimpacted stream sites (ecological quality class 5) in the analyses. A total of 64 sites were identified as being unimpacted and four of these sites were without growth of macrophytes.

The unimpacted sites in the STAR project were identified onsite by comparing site characteristics with a list of *a priori* exclusion criteria (Hering et al., 2003, Nijboer et al., 2004). In addition, pre-existing data on site conditions or GIS information were compared with the list of criteria for reference sites, when available. Table 1 gives an overview of the investigated unimpacted stream sites included in the analyses and their location in terms of ecoregion, country, latitude, longitude and altitude.

Macrophyte sampling

Macrophyte surveys were undertaken using the protocols associated with the Mean Trophic Rank (MTR) indexation method (Holmes et al., 1999). This method is the standard procedure used in the United Kingdom in association with the implementation of the European Union Urban Wastewater Directive and is compatible with methodologies used in several of the other Member States participating in STAR. The term

Table 1. An overview of the investigated unimpacted stream sites within each stream type and their location in terms of ecoregion, country, latitude and altitude

Stream type	Number of observations	Ecoregion	Country	Latitude (average)	Longitude (average)	Altitude (average)
Small-sized, shallow mountain streams	8	8, 9, 10	Austria, Czech Republic, Germany	49	13.9	399
Small-sized, lowland calcareous streams	3	18	United Kingdom	51	−1.6	46
Small-sized streams in the Central, sub-alpine mountains	3	9	Czech Republic	50	17.3	361
Small-sized, shallow headwater streams in Eastern France	3	8	France	48	5.4	344
Small-sized, calcareous mountain streams in Western, Central and Southern Greece	3	6	Greece	38	22.2	528
Small-sized Buntsandstein streams	2	9, 14	Germany	50	9.6	220
Small-sized, calcareous streams in the Central Apennines	3	3	Italy	43	11.4	393
Small-sized calcareous mountain streams in the Eastern Carpathians*	3*	10	Slovak Republic	49	22.3	413
Small-sized siliceous mountain streams in the Western Carpathians*	5*	10	Slovak Republic	49	18.6	408
Medium-sized, lowland streams	23	14, 15, 16, 18, 22	United Kingdom, Sweden, Poland, Denmark, Latvia	55	17.5	84
Medium-sized, lowland calcareous streams	3	18	United Kingdom	52	−2.7	119
Medium-sized streams in the lower mountainous areas of Southern Portugal	3	1	Portugal	39	−7.6	234
Medium-sized streams on calcareous soils	2	14	Sweden	60	17.8	9

*Two sampling sites were without growth of macrophytes in each of the two stream types.

macrophyte includes all higher plants that grow submerged or partly submerged, vascular cryptograms and bryophytes, together with groups of algae which can be seen to be composed predominantly of a single species. Therefore the term macrophyte also encompasses terrestrial species growing partly submerged in the stream channel. The sampling reach was 100 m in length. Macrophyte sampling was undertaken in late summer/early autumn 2002 or 2003. Macrophyte abundance was expressed in terms of the percentage of the survey length covered. A cover score was allocated to each macrophyte species present using the following scale 1: $<0.1\%$, 2: 0.1–1%, 3: 1–2.5%, 4: 2.5–5%, 5: 5–10%, 6: 10–25%, 7: 25–50%, 8: 50–75%, 9: $>75\%$. For all percentage cover estimates, the whole survey area surveyed equals 100%, i.e. the individual species percentage cover estimates are a percentage of the whole survey area and not of the overall percentage cover estimated. For wadeable surveys a glass-bottom bucket was used to aid observations. A grapnel was used to retrieve submerged macrophytes for identification from small areas of deep water. For non-wadeable areas a grapnel was used to retrieve macrophyte specimens from the banks. Particular care was taken to examine all small niches within the survey site to look for small patches of species. For a more detailed description, see Holmes et al. (1999) or the STAR website (http://www.eu-star.at) under the public-access section "Protocols". If identification to species could not be done due to absence of seasonal diagnostic features, e.g. *Ranunculus* and *Callitriche*, the record was only performed to the genus level (for species names and authors see Supplementary material).[1]

Site characteristics

The River Habitat Survey was also undertaken in late summer/early autumn 2002 or 2003 together with supporting chemical, physico-chemical and geographical elements. All relevant protocols, i.e. the AQEM and STAR site protocol, the river habitat survey (RHS) protocol and MTR protocol, are accessible at the STAR website (http://www.eu-star.at) under the public-access section "Protocols".

Data analysis

The pan-European taxonomic standardisation of the macrophyte data was used for all analyses performed (Furse et al., 2004). To analyse assemblage patterns in the *a priori* defined stream types a Detrended Correspondence Analysis (DCA) was performed (PC-ORD; McCune & Mefford, 1999) and DCA site scores were used to summarise the variability in assemblage patterns among the stream sites within each stream type. An Indicator Species Analysis (Dufrene & Legendre, 1997) was performed to identify indicator species (PC-ORD; McCune & Mefford, 1999). This analysis could only be performed for small-sized shallow mountain streams and medium-sized lowland streams, however, as the number of sampling sites was restricted to 2–3 in the other stream types. For each species encountered in the two stream types, an indicator value was calculated ranging from zero (no indication) to 100 (perfect indication). The indicator values were tested for statistical significance using a Monte Carlo permutation test. Only significant indicator species ($p < 0.05$) were used in data interpretation.

To further describe the variability within and among the stream type, mean values and ranges for a number of structural and ecological metrics were calculated. The structural metrics are mathematical expressions of community structure and the ecological metrics are based on the information of ecological tolerance of indicator species. In the present context the term macrophyte community is used broad and encompasses the complex of communities that may exist along the 100 m stream reaches studied.

The structural metrics used were species, genus and family richness, Shannon and Simpson diversity (Margalef, 1958) and domination and evenness. The index C that was used as a measure of domination was calculated as:

$$C = \sum_{i=1}^{S} \left(\frac{p_i}{\sum_{i=1}^{S} p_i^2} \right)^2$$

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[1] Supplementary material is available for this article at http://www.dx.doi.org/10.1007/s10750-006-0096-1 and accessible for authorised users.

where s is the number of species and p_i the abundance (share of the cover) of species i.

The index $E_{1/D}$ that was used as a measure of evenness was calculated as:

$$E_{1/D} = \frac{\left(1 - \sum_{i=1}^{S} \frac{N_i}{N}\right)}{S}$$

where S is the number of species, N the total abundance, and N_i is the abundance of species i.

In supplement to the above described diversity measures, species-area curves for the main stream types (i.e., small-sized, shallow mountain streams and medium-sized, lowland streams) were generated from the sample plots, and the overall species richness using the jackknife method was estimated (PC-ORD; McCune & Mefford, 1999).

The ecological metrics calculated were Mean Trophic Rank (MTR; Holmes et al., 1999) and Macrophytical Biological Index for Rivers (IBMR; Haury et al., 2002). These metrics are based on information of tolerance of species to eutrophication. MTR scores lie in the range 10–100, where low values (<25) indicate eutrophic conditions and values between 25 and 65 indicate either eutrophic conditions or that the site is at risk of becoming eutrophic (Holmes et al., 1999). IBMR was recently developed in France to assess water trophy and organic pollution in rivers. The IBMR scores vary between 0 (degraded) and 20 (high quality) (Haury et al., 2002). We did not statistically test for differences in DCA site scores or metric values among the *a priori* defined stream types because the number of sampling sites was low for most stream types invalidating the analysis.

Assemblage patterns were characterised independently from the *a priori* defined stream typology. A TWINSPAN classification of the 60 sampling sites was performed using default options in PC-ORD (McCune & Mefford, 1997). The significance of the classification was tested by comparing DCA coordinates among the major end-clusters (including more than six sites) using ANOSIM (Analysis of Similarities; Clarke & Green, 1988). We also calculated diversity and distributional metrics as well as ecological metrics (MTR and IBMR) for the major end-clusters.

An Indicator Species Analysis (Dufrene & Legendre, 1997) for the major TWINSPAN end-clusters was performed using cluster membership (cluster 1–8) as a grouping variable. The indicator values were tested for statistical significance as described above. The clusters were further characterised in terms of number of sampling sites present, their relation to the *a priori* defined types, species richness, dominant taxonomic groups, growth morphology and species abundance. The relative distribution of coverage classes was used as a measure of species abundance for the major end-clusters and these were tested statistically using a Kruskal–Wallis test. The distribution of species abundance was also evaluated using rank-abundance curves. The logarithm of the relative abundance of species was plotted as a function of the rank number (x) in each group. The rank number was scaled as x/S, where S is the number of species in the groups, so that the most abundant species had the lowest rank of $1/S$ close to zero, while the rarest species had the highest rank of 1 (Wilson, 1991).

The relationships between the major TWINSPAN end-clusters and stream site characteristics at various scales (ecoregion, catchment, riparian, habitat) were further analysed. In doing that an integrated measure of shade from riparian vegetation was calculated (weighted shade index, WSI). The WSI takes values in the interval [0; 200] and is defined as:

$$\mathrm{WSI} = \sum_{i=1}^{3} k_i \sum_{j=1}^{2} s_{ij},$$

where i is the degree of shading ($i=1$: no shading; $i=2$: 33%, $i=3$: greater than 33% shading) and j stands for left ($j=1$) or right bank ($j=2$). Finally, $k_1=0$, $k_2=25$ and $k_3=100$.

A variance analysis (ANOVA with Bonferroni correction) was performed to test for differences among the major end-clusters regarding macrophyte community characteristics and sampling site characteristics. Differences among categorical variables were tested using X^2. Relations between clusters and variables were analysed using Spearman rank correlation analysis. Some of the categorical variables, i.e. planform, flow category and water clarity, were treated as non-categorial variables in this analysis as the values assigned represented gradients. Thus increasing planform value implied increasing channel complexity

(1 = straight, 2 = sinuous, 3 = irregular meanders, 4 = regular meanders), increasing discharge values implied increasing discharge (1: < 0.31 m^3 s^{-1}; 2: >0.31–0.62 m^3 s^{-1}; 3: >0.62–1.25 m^3 s^{-1}; 4: >1.25–2.50 m^3 s^{-1}; 5: >2.5–5.0 m^3 s^{-1}; 6: >5.0–10.0 m^3 s^{-1}; 7: >10–20 m^3 s^{-1}; 8: >20–40 m^3 s^{-1}; 9: >40–80 m^3 s^{-1} and 10: >80 m^3 s^{-1}) and increasing clarity implied decreasing water clarity (1 = clear; 2 = cloudy; 3 = turbid). We chose to perform only correlation analysis to relate stream site characteristics to community variables as the environmental data for some of the variables were too incomplete to allow multivariate methods to be applied. Particularly the chemistry data were incomplete. The chemistry data from the sites in the Czech Republic were not included in the analysis as the detection limit was too high compared to detection limits in the other sites.

Results

Richness, diversity and metrics

Macrophytes were present in all *a priori* defined stream types, but the number of species, genera and families increased from small to middle-sized streams with the exception of small-sized, shallow headwater streams in Eastern France that were very species-rich (Table 2). Numbers of species, genera and families were all positively correlated ($r = 0.987$; $p < 0.05$). The jackknife estimates for overall species richness were 23 for small-sized, shallow mountain streams and 145 for medium-sized, lowland streams. The total number of species actually encountered was 14 in the small-sized, shallow mountain streams and 98 in medium-sized, lowland streams, which indicates that the number of investigated sampling sites of both stream types was too low to adequately estimate the average species richness.

Both the Shannon and Simpson diversity indices were also generally lower in the small-sized streams compared to the middle-sized streams (Table 2), again with the exception of small-sized, shallow headwater streams in Eastern France. The Shannon and Simpson diversity indices were positively correlated with the number of species, genera and families ($r = 0.973$; $p < 0.05$). The distributional indices, domination and evenness, also varied within and among the stream types. The domination index was negatively correlated with all indices ($r = -0.603$; $p < 0.05$), whereas the evenness index was unrelated to the other indices ($p > 0.05$).

The mean MTR was generally highest in the small-sized mountainous streams (58–80) except for the small-sized mountain streams in Western, Central and Southern Greece (45) (Table 2). The MTR was lower in the medium-sized mountainous streams (64) and lowest in the medium-sized lowland streams (37–46). In small-sized, shallow mountain streams the MTR varied between 50 and 100, and in medium-sized lowland streams the MTR varied between 28 and 79. The IBMR performed similarly to the MTR (Table 2). Both indices correlated negatively with the number of species, genera and families and with the diversity indices. The MTR correlated positively with the domination index. The IBMR and MTR indices were positively inter-correlated ($r = 0.586$; $p < 0.05$).

Macrophyte assemblage patterns

Some of the stream types showed a high degree of dispersion along the DCA ordination axes (small-sized, calcareous streams in the Central Apennines, medium-sized streams on calcareous soils), whereas other stream types were more uniformly distributed (small-sized streams in the Central, sub-alpine mountains, medium-sized lowland calcareous streams) (Figs 1a and b). The small-sized, shallow mountain streams were positioned in the middle of the ordination diagrams (Figs 2a and b). The medium-sized lowland streams were also positioned in the middle of the ordination diagrams, but to the left of small-sized, shallow mountain streams (Figs 1a and b). These sites were mainly distributed along DCA 2 (Fig. 2d). Only medium-sized, lowland stream sites from Sweden were clearly distinguishable from the other medium-sized, lowland stream sites (Germany, Latvia, Poland and Denmark) (Figs 1c and d). The medium-sized, lowland calcareous streams and the small-sized lowland calcareous streams were not clearly distinguishable from the other medium-sized, lowland stream sites (Figs 1a and b).

In total, 8 end-clusters were identified from the TWINSPAN classification (Fig. 2). Of these only

Table 2. Summary of the mean and ranges of various structural and ecological metrics in different *a priori* defined reference stream types

Stream type	Species number	Shannon diversity	Simpson diversity	Domination	Evenness	MTR	IBMR
Small-sized, shallow mountain streams	3 (1–6)	0.890 (0–1.792)	0.496 (0–0.833)	0.645 (0.166–1)	0.721 (0–1)	62 (50–100)	15 (13–18)
Small-sized, lowland calcareous streams	9 (1–14)	1.590 (0–2.480)	0.596 (0–0.907)	0.475 (0.157–1)	0.620 (0–0.940)	42 (38–46)	11 (11–11)
Small-sized streams in the Central, sub-alpine mountains	4.3 (4.0–5.0)	1.398 (1.311–1.550)	0.737 (0.716–0.776)	0.469 (0.383–0.632)	0.957 (0.946–0.963)	58 (50–62)	13 (10–15)
Small-sized, shallow headwater streams in Eastern France	33 (23–39)	3.279 (2.904–3.475)	0.951 (0.927–0.963)	0.195 (0.108–0.331)	0.938 (0.914–0.951)	42 (41–42)	11 (10–12)
Small-sized, calcareous mountain streams in Western, Central and Southern Greece	9 (4–14)	1.913 (1.332–2.375)	0.821 (0.720–0.891)	0.411 (0.272–0.633)	0.937 (0.925–0.961)	45 (43–47)	15 (14–16)
Small-sized Buntsandstein streams	7 (3–11)	1.673 (1.012–2.333)	0.756 (0.615–0.898)	0.366 (0.177–0.556)	0.947 (0.921–0.973)	60 (59–62)	10 (10–10)
Small-sized, calcareous streams in the Central Apennines	3 (2–5)	1.037 (0.693–1.519)	0.596 (0.500–0.757)	0.658 (0.500–0.862)	0.921 (0.819–1.000)	55 (45–70)	11 (9–13)
Small-sized calcareous mountain streams in the Eastern Carpathians	1						
Small-sized siliceous mountain streams in the Western Carpathians	1.3 (1–2)	0.230 (0–0.691)	0.166 (0–0.498)	0.844 (0.531–1.000)	0.332 (0–0.997)	80 (80–80)	15 (15–15)
Medium-sized, lowland streams	13 (2–32)	2.195 (0.693–3.330)	0.844 (0.500–0.959)	0.370 (0.102–0.831)	0.945 (0.860–1.000)	42 (28–79)	11 (9–14)
Medium-sized, lowland calcareous streams	12 (6–18)	2.289 (1.705–2.810)	0.877 (0.806–0.934)	0.252 (0.203–0.305)	0.956 (0.946–0.972)	37 (33–42)	10 (9–10)
Medium-sized streams in the lower mountainous areas of Southern Portugal	7 (5–8)	1.742 (1.359–2.007)	0.791 (0.680–0.858)	0.425 (0.206–0.841)	0.922 (0.845–0.965)	64 (60–70)	12 (12–12)
Medium-sized streams on calcareous soils	9 (2–16)	1.859 (1.040–2.678)	0.774 (0.625–0.924)	0.456 (0.279–0.633)	0.956 (0.946–0.966)	46 (45–47)	11 (11–11)

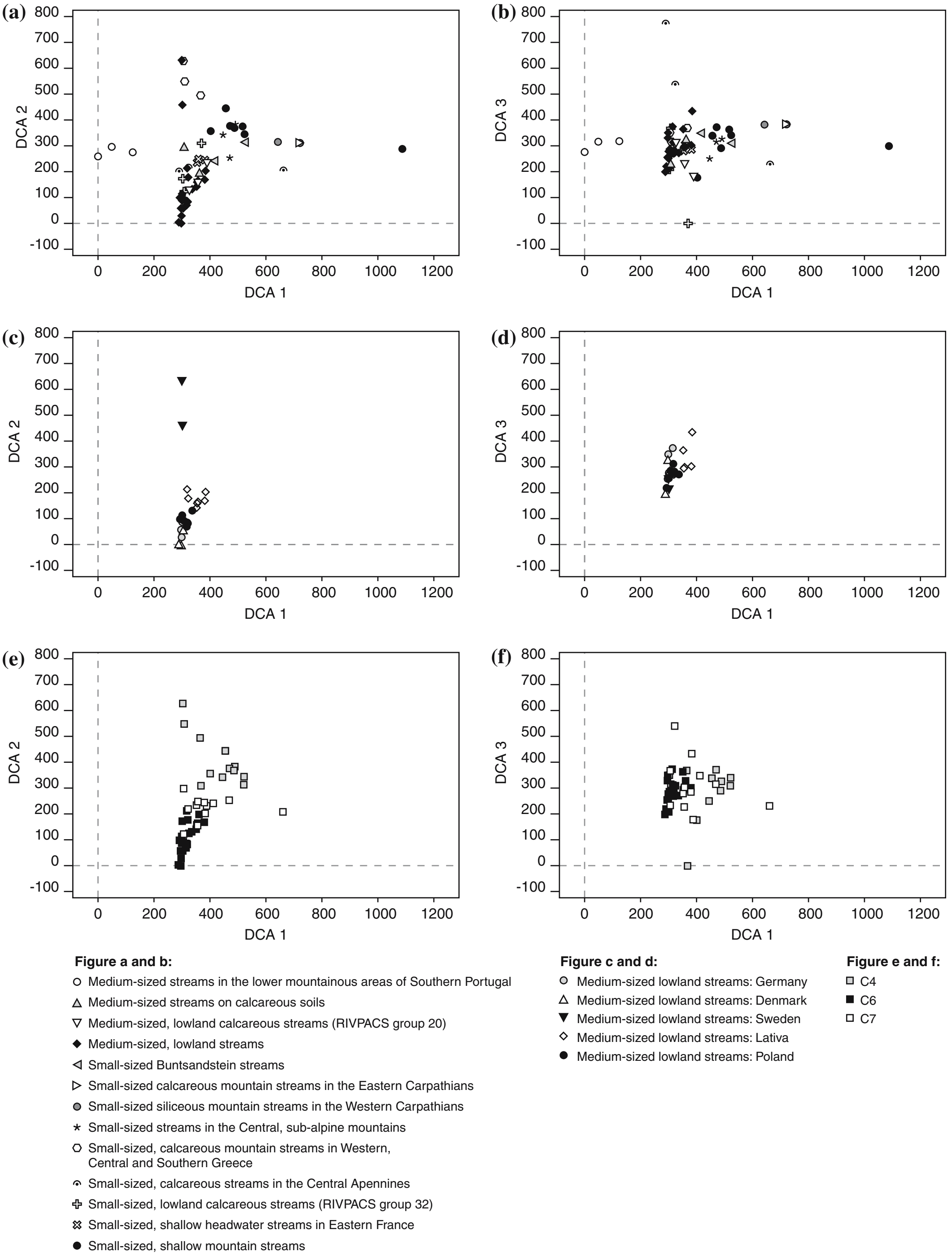

Figure 1. Detrended correspondence analysis (DCA) of 60 sample plots distributed in different streams situated all over Europe. The analysis was performed with downweighting of rare species. In total, 182 species were included in the analysis. (a) and (b) include all sampling sites and different symbols are used for different *a priori* defined stream types. (c) and (d) include medium-sized, lowland streams sites with different symbols for different countries. E and F include C4, C6 and C7 sites identified from the TWINSPAN classification of sampling sites.

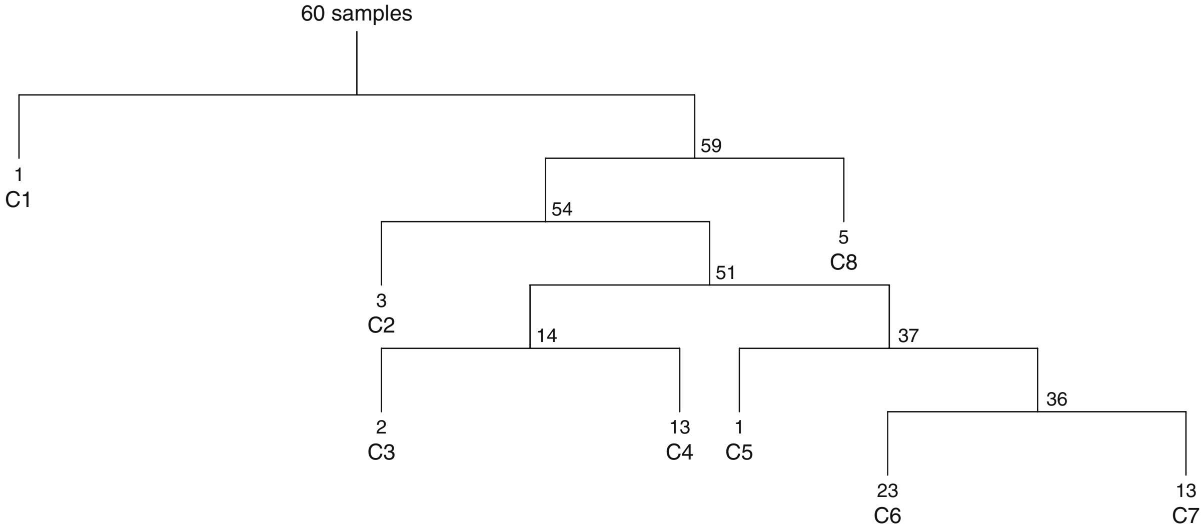

Figure 2. TWINSPAN tree with 8 end-clusters. The number of samples is shown above each node. End-clusters are named C1 to C8. Only C4, C6 and C7 ($n > 6$) were subjected to further analyses.

three end-clusters (C4, C6 and C7) were sufficiently large (>6 sites) for subsequent analysis. Species abundance and indicator values for the three end-clusters are given in Supplementary material. A large group of small-sized streams (C4) consisted of several of the *a priori* defined small-stream types (Table 3). This end-cluster was relatively species-poor with predominant growth of mosses (e.g., *Rhynchostegium riparioides* and *Cratoneuron filicinum*) (Table 3; Supplementary material). A large group of medium-sized lowland streams (C6) was species-rich and consisted of primarily vascular plants (e.g., *Sparganium emersum*, *Phalaris arundinacea*, *Berula erecta* and *Elodea canadensis*) (Table 3; Supplementary material). Finally, a mixed group of small- and medium-sized streams (C7) displayed intermediate characteristics. As opposed to C4 this end-cluster was very species-rich with growth of both mosses (e.g., *Fontinalis antipyretica*) and many amphibious (*Veronica anagallis-aquatica* and *Myosotis palustris*) and terrestrial dicots (Table 3; Supplementary material).

End-cluster C4, C6 and C7

The three end-clusters C4, C6 and C7 were found to differ significantly based on their DCA coordinates (ANOSIM, $p < 0.05$; Figs 1e and f). The number of species, genera and families encountered, and diversity indices also varied among the three clusters (Fig. 4; ANOVA $p < 0.05$). C4 had fewer species, genera and families compared to C6 and C7, and the Shannon and Simpson diversity were also lower. In contrast, the domination index was higher for this cluster (Fig. 4; ANOVA $p < 0.05$). This was mainly related to a high abundance of *Rhynchostegium riparioides* in many C4 sites. C6 and C7, on the other hand, were very similar regarding species, genera and family richness as well as diversity and domination indices (ANOVA $p > 0.05$), but C7 possessed a distinct community that shared characteristics with both small-sized, shallow mountain streams and middle-sized, lowland streams (Table 3; Supplementary material). The MTR also varied among C4, C6 and C7 (Fig. 4). C4 had the highest and most variable MTR scores and this cluster was clearly distinguishable from C6 and C7 (ANOVA $p < 0.05$). In contrast, C6 and C7 had more similar and less variable MTR scores. The IBMR scores gradually declined from C4 to C6 and C7 (ANOVA $p < 0.05$).

There was a high degree of overlap between the main stream types, i.e., small-sized shallow mountain streams and medium-sized lowland streams and end-cluster 4 and 6 (Figs 1e and f and Table 3). This was reflected in a high percentage of

Table 3. Characteristics of the TWINSPAN end-clusters and their relations to the *a priori* defined stream types

> Identified clusters	Typology	Number of species	Indicator species: small-sized, shallow mountain streams	Indicator species: medium-sized, lowland streams	Dominant group	Dominant growth morphology	DCA score axis 1	DCA score axis 2	DCA score axis 3
End-cluster 1	Small-sized shallow mountain streams (1)	5	0	0	Moss, algae	Submerged	1066 (860–1118)	280 (204–299)	281 (101–326)
End-cluster 2	Medium-sized streams in the lower mountainous areas of Southern Portugal (3)	14	0	0	Dicots, monocots	Submerged, terrestrial	82 (−47–291)	279 (96–540)	307 (196–356)
End-cluster 3	Medium-sized lowland streams (Sweden) (2)	12	2	1			299 (290–332)	603 (289–751)	237 (180–344)
End-cluster 4	Small-sized shallow mountain streams (6) Small-sized, lowland calcareous streams (1) Small-sized streams in the Central, sub-alpine mountains (2) Small-sized, calcareous mountain streams in Western, Central and Southern Greece (3) Small-sized Buntsandstein streams (1)	17	2		Moss, liverworts	Submerged	377 (289–315)	464 (263–613)	300 (0–427)
End-cluster 5	Small-sized, calcareous streams in the Central Apennines (1)	3	0	0	Algae	Submerged	290	205	777
End-cluster 6	Medium-sized, lowland streams (20) Medium-sized, lowland calcareous streams) (2) Medium-sized streams on calcareous soils (1)	69		11	Dicots	Amphibious, submerged	305 (260–381)	56 (−203–227)	272 (−79–539)
End-cluster 7	Small-sized, lowland calcareous streams (1) Medium-sized, lowland calcareous streams (2) Small-sized streams in the Central, sub-alpine mountains (1) Small-sized, shallow headwater streams in Eastern France (3) Small-sized Buntsandstein streams (1) Small-sized, calcareous streams in the Central Apennines (2) Medium-sized streams on calcareous soils (1) Medium-sized, lowland streams (1)	63		2	Moss, dicots	Submerged, amphibious	351 (286–860)	236 (6–490)	295 (50–844)
End-cluster 8	Small-sized shallow mountain streams (1) Small-sized calcareous mountain streams in the Eastern Carpathians (2) Small-sized siliceous mountain streams in the Western Carpathians (3)	2	1	0	Liverworts, moss	Submerged	644 (566–722)	316 (312–320)	383 (382–384)

The number of sites of the *a priori* defined stream types within each end-cluster is given in brackets.

overlap between the sites of 75% and 87%, respectively, and in an overlap of indicator species identified for small-sized, shallow mountain streams and C4 (*Rhynchostegium riparioides*) and for medium-sized, lowland streams and C6 (e.g., *Sparganium emersum*, *Berula erecta*, *Elodea canadensis*) (Table 3). There was no indicator species overlap between C4 and medium-sized, lowland streams, or between C6 and small-sized, shallow mountain streams. End-cluster 7 was a very broad group and eight *a priori* defined stream types were represented in this cluster (Table 3). The identified indicator species for this end-cluster included both species identified as indicator species for small-sized, shallow mountain streams and for medium-sized, lowland streams (*Veronica anagallis-aquatica* and *Myosotis palustris*) (Table 3; Supplementary material). Two of the most abundant species (*Hygroamblystegium fluviatile* and *Fontinalis antipyretica*) were also found in both small-sized, shallow mountain streams and medium-sized, lowland streams.

The species occurring in the identified clusters were all very low in abundance (Fig. 3a) and the distribution of coverage classes for the three clusters did not vary significantly (Kruskal–Wallis, $p > 0.05$). Distribution of species abundance in C4, C6 and C7 was further evaluated using rank-abundance curves (Fig. 3b). *Rhynchostegium riparioides* was very abundant in C4 sites, counteracting the general abundance pattern.

End-cluster C4, C6 and C7 and stream site characteristics

Several stream site characteristics differed among end-cluster C4, C6 and C7 (Table 4; ANOVA, $p < 0.05$; χ^2, $p < 0.05$). In general, C4 and C6 were very distinct as to both ecoregion, catchment, riparian and habitat variables, whereas C7 shared characteristics with both C4 and C6 (Table 4). C4 sites, in particular, were positioned at higher altitudes and lower latitudes, they had steeper slopes (ANOVA $p < 0.05$) and were positioned closer to the source compared to C6 sites (ANOVA $p < 0.05$). C4 sites were also less wide and more shaded from riparian vegetation compared to C6 sites, and with predominantly alluvial deposits in the valley (Table 4). C7 sites were at higher altitudes than C6 sites, they were closer to the source, less wide, and moraine deposits were less widespread in the valleys compared to C6 sites (Table 4).

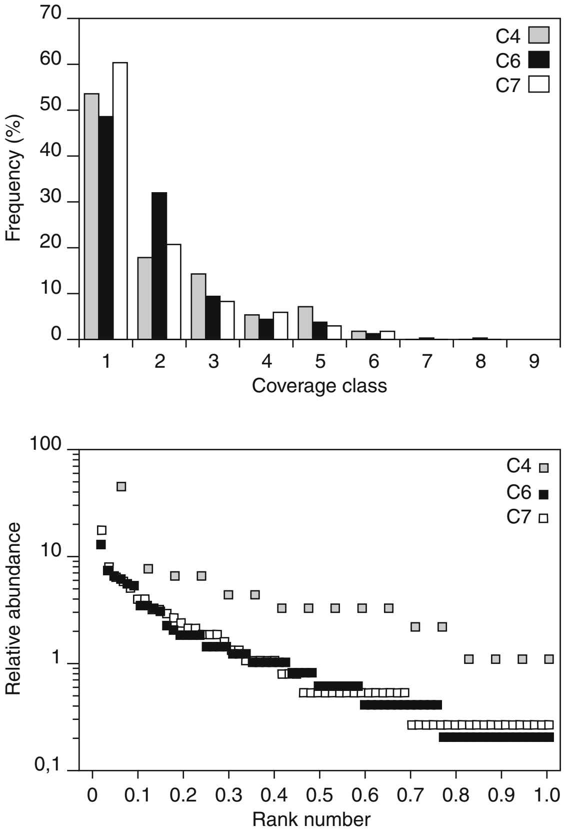

Figure 3. (a) Distribution of coverage classes expressed as the relative frequency of coverage class 1–9 divided by the total number of species allocated to a coverage class in end-cluster C4, C6 and C7 respectively. The coverage classes were 1: <0.1 %, 2: 0.1–1%, 3: 1–2.5%, 4: 2.5–5%, 5: 5–10%, 6: 10–25%, 7: 25–50%, 8: 50–75%, 9: >75%. (b) Rank-abundance curves expressing the relative distribution of species abundance according to their rank. The relative abundance was calculated as the sum of coverage classes allocated to a species divided by the total sum of coverage classes in end-cluster C4, C6 and C7, respectively.

The variability in the calculated metrics was slightly higher in the *a priori* defined middle-sized, lowland streams compared to the corresponding end-cluster C6 (Table 5) and also in the small-sized, shallow mountain streams compared to the end-cluster C4 with the exception of IBMR and species number. The variability in MTR, in particular, was higher in the *a priori* defined stream types compared to the corresponding TWINSPAN end-clusters (Table 5).

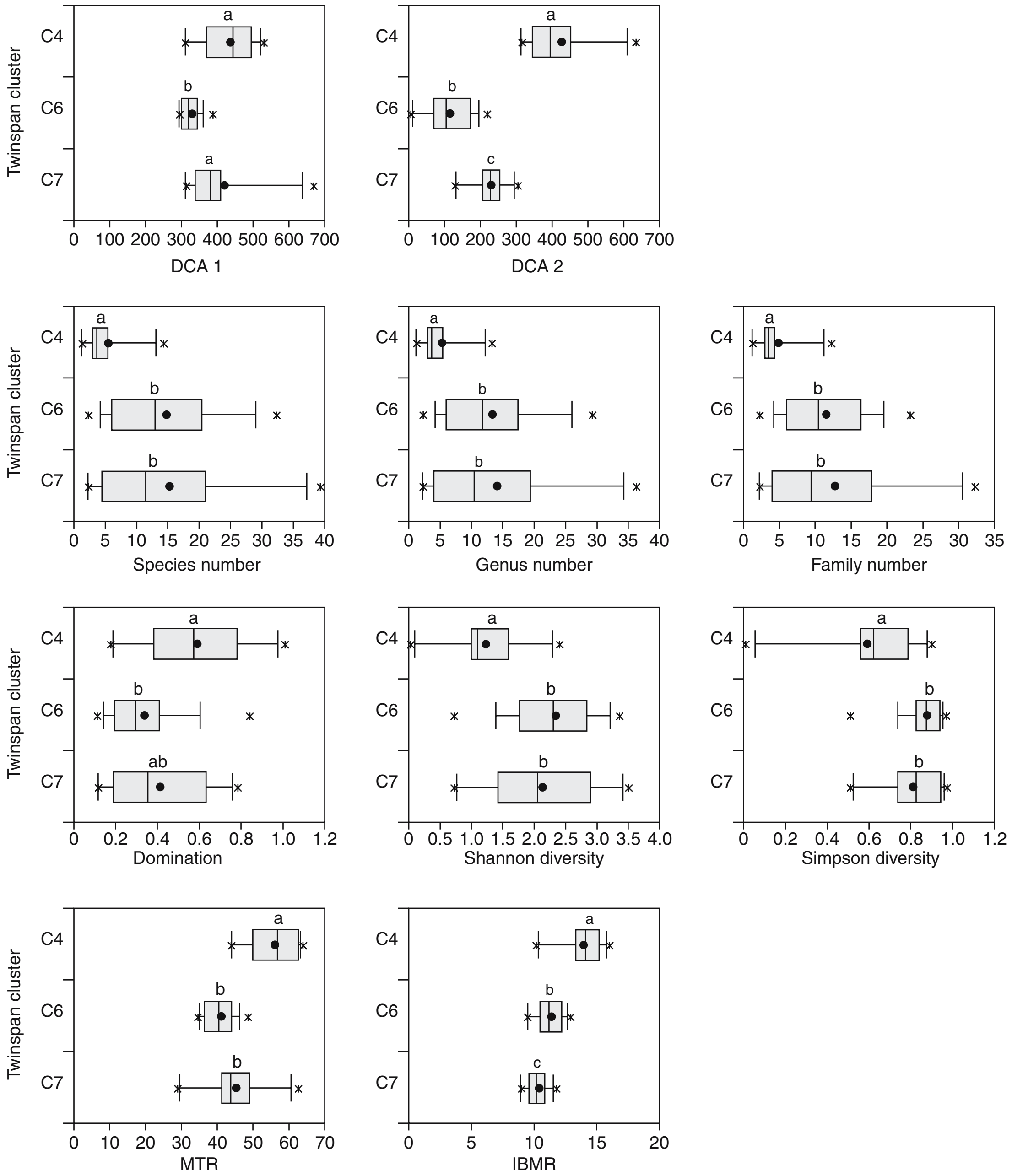

Figure 4. Box-whisker plots of macrophyte community characteristics in end-clusters C4, C6 and C7. Letters a, b and c signify differences between mean values (ANOVA with Bonferroni correction, $p < 0.05$). The box represents 10, 25%, median, 75 and 90% and the symbol the mean value. Error bars represent the 5% and 95% and the star (*) the minimum and maximum value.

Significant correlations were found between many macrophyte community characteristics (i.e., DCA scores, richness, diversity, MTR and IBMR) and stream site characteristics at various scales (Table 6). The separation on the DCA axis was mainly related to ecoregion, catchment and riparian variables (e.g., altitude, slope, discharge, and catchment area). Habitat variables were mainly of

Table 4. Ecoregion, catchment, riparian and habitat characteristics of the major TWINSPAN end-clusters 4 ($n = 13$), 6 ($n = 23$) and 7 ($n = 12$)

	Cluster 4		Cluster 6		Cluster 7	
	Mean/Median	SD	Mean/Median	SD	Mean/Median	SD
Ecoregion variables						
Latitude	43.3^{a}	14	54.7^{b}	2.4	50.3^{b}	5.1
Altitude (m)	379.9^{a}	205.4	71.7^{b}	44.9	221.6^{c}	144
Catchment variables						
Slope (m km^{-1})	42.8^{a}	54.6	4.2^{b}	7.6	5.5^{b}	6.3
Distance to source (km)	10.4^{a}	4.4	31.2^{b}	16.1	16.9^{a}	9.3
Height of source (m)	769.8^{a}	406.2	137.4^{b}	77.9	421.5^{c}	252.8
Catchment area (km^2)	26.7^{a}	15.0	179.9^{b}	119.0	89.8^{a}	73.8
Riparian variables						
Drift geology*$^{1)}$	1^{a}		3^{b}		1^{ab}	
Planform*$^{2)}$	2^{a}		3^{b}		3^{b}	
Habitat variables, physical						
Width (m)	4.41^{a}	2.03	8.23^{b}	3.27	5.86^{ab}	2.04
Discharge*$^{3)}$	1^{a}		4^{b}		3^{b}	
Clarity*$^{4)}$	3^{a}		2^{b}		3^{ab}	
Shading – integrated measure $^{5)}$	171.2^{a}	70.6	96.7^{b}	71.6	116.7^{b}	82.8
Habitat variables, chemical						
Chloride (mg l^{-1})	6.5^{a}	3.6	29.1^{b}	12.2	15.7^{a}	7.6
BOD5 (mg l^{-1})	2.1	1.1	1.5	1.0	1.6	0.8
Ortho-phosphate (mg l^{-1})	24.3	11.5	14.2	13.9	8.5	1.7
Total phosphate (mg l^{-1})	42.9^{a}	16.2	28.8^{ab}	8.8	18.0^{b}	6.0

(1) Drift geology: 1: alluvial, 2: lacustrine, 3: moraine, 4: sandar, 5: marine, 6: organic.(2) Planform: 1 = straight; 2 = sinuous; 3 = meanders, irregular; 4 = meanders, regular.(3) Discharge: 1: $< 0.31\ \text{m}^3\ \text{s}^{-1}$, 2: > 0.31–$0.62\ \text{m}^3\ \text{s}^{-1}$, 3: > 0.62–$1.25\ \text{m}^3\ \text{s}^{-1}$, 4: > 1.25–$2.50\ \text{m}^3\ \text{s}^{-1}$, 5: > 2.5–$5.0\ \text{m}^3\ \text{s}^{-1}$, 6: > 5.0–$10.0\ \text{m}^3\ \text{s}^{-1}$, 7: > 10–$20\ \text{m}^3\ \text{s}^{-1}$, 8: > 20–$40\ \text{m}^3\ \text{s}^{-1}$, 9: > 40–$80\ \text{m}^3\ \text{s}^{-1}$ and 10: $> 80\ \text{m}^3\ \text{s}^{-1}$.(4) Water clarity: 1 = clear; 2 = cloudy; 3 = turbid.(5) Shade: see Data analysis section for formula.Only variables that differed among clusters are included (ANOVA $p < 0.05$ or X^2 for categorical variables see*). a, b and c signifies differences between mean/median values ($p < 0.05$).

significance for the separation of C6 and C7 sites on DCA axis 2 (Figs 1e and f, Table 6). Richness of species, genera and families were negatively related to slope and positively to discharge, increasing channel complexity and stream width. The Shannon diversity was also negatively related to slope and to shading, whereas it was positively related to stream width (Table 6). The MTR was also correlated to many variables at various scales (e.g., altitude, slope, distance to source, discharge). The IBMR was less correlated to the ecoregion variables than MTR but to several of the habitat variables (e.g., depth, substrate type). The correlation between IBMR and ortho-phosphate is based on only 13 measurements and the found relation should therefore be considered with caution.

Discussion

General patterns in community structure

We found that macrophytes were present in almost all the investigated stream and river types but also found that their abundance was limited, which probably relates to unfavourable habitat conditions (i.e., high disturbance levels in upland regions and shading from riparian vegetation in both upland and lowland regions). We also found that there was a high degree of variability in community structure among the main stream types investigated. Moving from the small streams in upland areas (small-sized, shallow mountain streams) to middle-sized lowland streams there was a clear

Table 5. Percentage coefficient of variation (CV) for various metrics describing structural and ecological characteristics of the macrophyte community for small-sized, shallow mountain streams and middle-sized lowland streams and for the corresponding TWINSPAN end-cluster 4 and 6

	CV%			
	Small-sized, shallow mountain streams ($n = 8$)	TWINSPAN C4 ($n = 13$)	Middle-sized, lowland streams ($n = 23$)	TWINSPAN C6 ($n = 23$)
MTR	27	23	23	10
IBMR	11	12	12	9
Species number	59	80	70	63
Shannon diversity	72	68	32	29
Simpson diversity	64	59	13	12
Domination	58	49	57	56
Evenness	62	57	4	3

transition from a predominance of species-poor mosses and liverwort-dominated communities to more species-rich communities dominated by vascular plants (Tables 2, 3 and Fig. 1). We could not clearly distinguish among the various different small-sized or medium-sized stream types on the basis of the macrophyte community structure (Fig. 1 and Table 2). This result may indicate that the number of sites investigated within the stream types was too low to give an adequate description of the macrophyte communities, or that the typology used is unsuited to describe macrophyte assemblage patterns (discussed later).

To characterise assemblage patterns independently from the typology a TWINSPAN classification was performed. Three distinct groups of plant species were identified (C4, C6 and C7, see Supplementary materials) of which two turned out to largely support two of the pre-defined main typologies. Thus there was a very good agreement between small-sized, shallow mountain streams and end-cluster C4 and between medium-sized, lowland streams and end-cluster C6 (75 and 87%, respectively). The last major end-cluster, C7, possessed a distinct community that shared characteristics with both the small-sized, shallow mountain streams and the middle-sized lowland streams. This community can be characterised as an intermediate community with growth of both many different species of non-vascular plants (e.g., *Fontinalis antipyretica*, *Amblystegium riparium*, *Fissidens carssipes*) and vascular plants (e.g., many amphibious and terrestrial species).

The result of the classification performed has many similarities with that previously performed in Great Britain (Holmes, 1998). Holmes identified four groups (A–D) based on the classification of more than 1500 British stream and river sites. The C6 end-cluster identified in this study is comparable to his A group, which was defined as eutrophic lowland streams comprising both low gradient lowland rivers, clay-dominated lowland rivers, chalk rivers and other base-rich rivers with stable flows and finally, impoverished lowland rivers. The C6 end-cluster was species-rich and contained many more truly aquatic species than the other clusters. Similarly to Holmes (1998), we also found that some of the calcareous stream sites were located within this cluster (i.e., medium-sized, lowland calcareous streams and medium-sized streams on calcareous soils). The C7 end-cluster was comparable to the B group identified by Holmes (1998). The B group comprises sandstone, mudstone and hard limestone rivers of England, Wales and Scotland. In our study the C7 end-cluster also comprised streams sites with varying geology (Table 3). In this cluster the submerged habitats were often dominated by non-vascular plants, whereas a wide array of vascular amphibious and terrestrial species grew emergent in the stream channel. The C4 end-cluster was not really comparable to the other groups defined by Holmes (1998), as non-vascular species were more predominant in this cluster compared to the two remaining groups identified in Great Britain. Holmes (1998) performed further sub-divisions of

Table 6. Significant Spearman rank correlation coefficients between various macrophyte community characteristics and stream site variables at different scales: ecoregion, catchment, riparian and habitat for TWINSPAN end-clusters 4, 6 and 7

Variables	Number of observations	DCA1	DCA2	Species number	Shannon	Domination	Evenness	MTR	IBMR
Ecoregion variables									
Latitude	44–48	−0.384**	−0.641***					−0.418**	
Altitude (m)	44–48	0.549***	0.674***					0.469**	
Catchment variables									
Slope (m km^{-1})	42–46	0.467**	0.609***	−0.443*	−0.467*	−0.492*	−0.325*	0.675***	0.490**
Distance to source (km)	44–48		−0.587***	0.390*	0.406*	−0.361*		0.573***	
Height of source (m)	44–48	0.590***	0.740***	−0.285*				0.433*	
Discharge$^{(1)}$	38–41	−0.450*	−0.529**	0.617***	0.635***			−0.526**	
Catchment area	29–33	−0.477*	−0.666***	0.452*	0.465*			0.656***	
Riparian variables									
Planform (1–9)$^{2)}$	44–48		−0.356*	0.396*	0.411*	−0.324*			
Shading – integrated measure$^{(3)}$	44–48	0.353*				0.398*			
Habitat variables, physical									
Clarity $^{(4)}$	43–47	0.308*				0.398*		0.315*	
Width (m)	44–48		−0.495**	0.458**	0.472*	0.404*		−0.431*	
Bed stability, unstable (%)	43–47		−0.347*						
Bed stability, stable (%)	43–47		0.338*						
Depth: 0.25–0.50 (%)	43–47		0.328*						
Depth > 1.0 (%)	43–47								0.318*
Substrate: Bedrock (%)	44–48							−0.417*	−0.400*
Substrate: Boulders/copples (%)	44–48	0.341*	0.350*						
Habitat variables, chemical									
Chloride (mg l^{-1})	21–24	−0.521**	−0.810***					−0.663**	
Nitrate (mg l^{-1})	10–11		−0.694*						
Ortho-phosphate (mg l^{-1})	12–13								0.681*

(1) Discharge: 1: <0.31 m^3 s^{-1}, 2: >0.31–0.62 m^3 s^{-1}, 3: >0.62–1.25 m^3 s^{-1}, 4: >1.25–2.50 m^3 s^{-1}, 5: >2.5–5.0 m^3 s^{-1}, 6: >5.0–10.0 m^3 s^{-1}, 7: >10–20 m^3 s^{-1}, 8: >20–40 m^3 s^{-1}, 9: $>$ 40–80 m^3 s^{-1} and 10: >80 m^3 s^{-1}.(2) Planform: 1 = straight; 2 = sinuous; 3 = meanders, irregular; 4 = meanders, regular.(3) Shade: see Data analysis section.(4) Water clarity: 1 = clear; 2 = cloudy; 3 = turbid. *$p < 0.05$. **$p < 0.001$. ***$p < 0.0001$.

the identified groups into 38 sub-types. Many of these sub-types are not or only poorly represented in the present investigation and therefore we will not compare the two classifications at a more detailed level.

As stated above, the general trend moving from C4 to C6 over C7 relates to a gradient from high altitude, high gradient, and small stream sites with steep slopes to lower altitude small and medium-sized stream sites (Tables 4 and 6). Thus there was a clear gradation of mean site altitude with C4 sites being 81% higher than C6 sites, and C7 sites being 42% higher than C6 sites. Altitude has also previously been identified as being of primary importance for the distribution of plant communities in European streams (Haslam, 1987). Particularly mosses and liverworts that were predominant in C4 sites (see Supplementary material) were abundant in these streams, probably reflecting their preference for stable substrates and low water depths (Haslam, 1987; Scarlett & O'Hare, 2006). This was also evident from the correlations found between DCA axes and both streambed stability and the predominance of coarse substrata, i.e. boulders/cobbles (Table 4). The C7 sites were intermediate altitude streams that were positioned closer to the source compared to C6 sites. The C7 sites can be considered intermediate between strictly upland stream sites and lowland sites (Haslam, 1987; Holmes, 1998). This group consisted of a mixture of mosses and vascular plants (see Supplementary material). We found that amphibious species were an important feature of this group compared to C4 and C6, which probably relates to the stream size. Thus amphibious species tended to be most abundant in shallow water and the relative abundance of this group of species will therefore decrease from upstream to downstream reaches as the water depth increases (Riis et al., 2001). Finally, the C6 sites were true lowland sites with moraines deposits in the valley in several of the investigated sites and soft streambeds. These sites were unique in being dominated by vascular aquatic plant species (see Supplementary material) of which four were identified as indicator species, namely *Sparganium emersum*, *Berula erecta*, *Elodea canadensis* and *Lemna minor*.

Several smaller-scale habitat variables also affected the segregation of the macrophyte communities (Table 6), but many of these co-correlated with the large-scale variables mentioned above. However, shading and water clarity were important small-scale variables (Table 6). Thus moving from C4 over C7 to C6 sites the shading got less intense, which is likely to relate to the wider stream reaches that diminish the degree of shading from the riparian vegetation.

Metrics and their variation within C4, C6 and C7

The here-performed analysis suggests that great care should be taken in comparing macrophyte-based metrics in an evaluation of ecological quality *sensu* WFD without a detailed knowledge of stream type characteristics. This concerns both metrics that are mathematical expressions of the community structure (based on taxa richness, evenness and abundance patterns) and metrics based on the information of ecological tolerance of indicator taxa. We found that both types of metrics exhibited an intrinsic variability among the community types identified. The C4 community was less diverse (both richness and diversity measures) than the C6 and C7 communities, and the domination of a single or a few species was more typical here than in the other stream types. Similarly, the MTR and IBMR indices, both developed as assessment methods of the trophic status of streams and rivers, exhibited a marked variability among the three community types. The C4 community exhibited higher MTR scores (43–63) than the C6 (34–48) and C7 community (28–62). This result indicates that a natural shift in macrophyte abundance patterns takes place moving from upland to lowland sites (discussed below). This result also emphasises the general recommendation that the use of MTR should be restricted to making comparisons between streams and rivers that are of the same physico-chemical type (Dawson et al., 1999). Otherwise the lower MTR scores in C6 and C7 sites observed can incorrectly be inferred as more enriched conditions compared to C4 sites.

We found that much of the above-mentioned variation in metrics can be ascribed to differences in the physical stream environment among the identified types moving from upland to lowland regions (Table 6). Thus a low species-richness and diversity seems to be an inherent feature of small stream reaches as both parameters were negatively correlated with slope and discharge. Similarly we found that the MTR and IBMR were positively correlated with these environmental parameters, which suggests that these indices will be higher in upland reaches compared to wider lowland reaches with lower flow velocities. This is not surprising as species that typically grow in upland reaches, i.e., mosses and liverworts are high-scoring MTR species (Dawson et al., 1999). Thus the median STR (Species Trophic Rank), which is a value assigned to a species on a scale from 1 to 10 designed to reflect the tolerance of that species to eutrophication, is 8, 10 and 5 for liverworts, mosses and vascular plants, respectively (Dawson et al., 1999).

Species richness was associated with higher discharges and wider reaches that are less shaded from riparian vegetation. The increase in species richness with increasing stream size confirms the general positive correlation between species richness and area (Rosenzweig, 1995). In addition, the increase in species richness probably relates to habitat characteristics (French & Chambers, 1996). Thus, middle-sized streams are likely to be physically more heterogeneous and experience lower levels of disturbance than small-sized streams, which may promote the co-existence of a wider array of species (Vannote et al., 1980). In addition, when moving from upstream to downstream reaches a continuously larger upstream area is likely to enhance the propagule supply with the current and thereby species recruitment locally, which may also increase species richness (Barrat-Segretain, 1996).

Conclusions

We found that macrophyte communities in unimpacted European streams responded to most of the characteristics underlying the typological framework defined in the EU Water Framework Directive (WFD: European Commission, 2000). The present interpretation of the WFD typology may not, however, be adequate for an evaluation of stream quality based on macrophytes. First and most important, by using this typology we may overlook an important community type (C7), which is characterised as small-sized relatively steep-gradient streams being an intermediate type between small-sized, shallow mountain streams and medium-sized, lowland streams. This stream type is species-rich and consists of a mixture of non-vascular and vascular plant species. Second, the natural variability in most structural and ecological metrics appeared to be higher when using the pre-defined typology compared to a typology based on macrophyte assemblage patterns particularly regarding MTR. The consistency of these results should be examined by analysing a larger number of sites. Particularly the need of re-defining the typology to improve the ability to detect impacts in streams and rivers from macrophyte assemblage patterns should be investigated.

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