**Taxonomic and functional successional patterns in
macroinvertebrates related to flying dispersal abilities: a case
study from isolated manmade ponds at reclaimed opencast
coal mines**

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Abstract It has been proposed that communities change from *r* to *K* strategies during primary succession. However, because strong-flying organisms are expected to arrive first in newly created habitats and they show trait characteristics associated more often with *K* strategies, we hypothesized that the *r* to *K* trajectories would be more closely followed by flightless and poorly-flying organisms. Moreover, we expected that macroinvertebrate communities would converge in their functional composition due to deterministic forces while diverging as taxonomic assemblages due to stochastic drift and biotic interactions. However, we also expected that dispersal abilities of the organisms would affect these tendencies. To address these questions, macroinvertebrates were sampled from isolated manmade ponds of different ages (1-22 years old) constructed at reclaimed opencast coal mines. In accordance with our expectations, only flightless and poorly-flying organisms exhibited a slight shift from *r* to *K* strategies, the community taxonomically diverged along the primary succession gradient, and stochastic drift showed greater effects on strong-flying organisms. In contrast, the community did not converge in its functional composition. The weak differences observed among the

26 macroinvertebrates from ponds of different ages suggested that limiting environmental
27 conditions prevented the organisms from evolving to a more structured community.

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30 **Key words** primary succession, macroinvertebrates, fly, dispersal, biological traits, taxonomic
31 composition, deterministic forces, stochasticity

32

33 **Introduction**

34 Succession dynamics is a key conceptual process in community ecology (Margalef, 1968;
35 Odum, 1969; Gutiérrez & Fey, 1980), but surprisingly, information concerning the process of
36 primary succession in communities of aquatic macroinvertebrates is still scarce (Flory &
37 Milner, 2000; Cañedo-Argüelles & Rieradevall, 2011). Newly created habitats (i.e., manmade
38 ponds) have proven to be ideal systems for testing temporal community dynamics (Noon, 1996;
39 Hassall et al., 2012; Ruhí et al., 2013), and may be of especial relevance for analyzing primary
40 succession. However, the monitoring of these new ecosystems usually did not reach beyond 5
41 years in duration, and most succession research has been based on the study of temporary
42 wetlands, so that secondary successional processes were analyzed (e.g., Lake et al., 1989;
43 Velasco et al., 1993; Boix et al., 2004).

44 Traditionally, it has been proposed that if an ecological vacuum is filled (e.g., construction
45 of a manmade pond) selection will shift a community from r toward the endpoint K , from
46 generalist to more specialist taxa (MacArthur & Wilson, 1967; Townsend & Hildrew, 1994). At
47 the initial stages of succession, organisms of smaller sizes, with shorter life cycles, and good
48 dispersal abilities are expected to be more abundant, while at advanced stages of succession,
49 increases in the organisms' body sizes, life cycle durations, and the incidence of passive
50 dispersal would be expected. Small-bodied organisms often correlate with short life cycles
51 (Southwood, 1977; Townsend & Hildrew, 1994; Bêche et al., 2006), a high tolerance to adverse
52 conditions (Townsend & Hildrew, 1994; Townsend et al., 1997; Verberk et al., 2008) and the
53 occupation of lower trophic positions (Woodward & Hildrew, 2002; Brown et al., 2004;
54 Jonsson et al., 2005). During the colonization of isolated water bodies, aerial active dispersal
55 may have an important role in the community assemblages. In freshwater macroinvertebrates,
56 aerial active dispersal results predominantly from the flight of adult insects, but not all the
57 flying insects have the same flying abilities (Bilton et al., 2001). Strong-flying
58 macroinvertebrates show characteristics opposite to those predicted for r organisms by the r/K
59 theory (Harrison & Dobson, 2008): the orders of Coleoptera, Hemiptera and Odonata, with

60 better flight and dispersal abilities, are typically large and brightly colored, live relatively long
61 adult lives and are frequently the top predators in aquatic systems (i.e., fitting more the K -
62 strategy); while Ephemeroptera, Plecoptera, Trichoptera and Diptera, with poor power of flight,
63 are typically small, short-lived, cryptically colored, non-predatory taxa (i.e., fitting the r -
64 strategy). Consequently, if we analyze the functional composition of the community during
65 primary succession based on their dispersal abilities, on the one hand, the better flying
66 organisms rarely are going to follow the expected trend from r to K strategies because they are
67 mainly composed by organisms with K characteristics. On the other hand, the heterogenic group
68 formed by poorly flying insects (e.g. Diptera and Trichoptera) to non flying macroinvertebrates
69 (e.g. Gastropoda and Oligochaeta) have higher probabilities of follow the predicted changes by
70 the r/K theory because the poorly flying insects were closer to the predicted r characteristics.

71 The issue of whether the structure of ecological communities is deterministic or historically
72 contingent (from stochastic drift and biotic interactions) has become increasingly controversial
73 (Samuels & Drake, 1997; Belyea & Lancaster, 1999; Chase, 2003). The convergence of
74 biological communities has been related to deterministic forces rooted in the climax concept of
75 succession (Clements, 1916). In other words, communities that develop under similar conditions
76 would converge on common structures (Samuels & Drake, 1997; Fukami & Wardle, 2005). In
77 this sense (Jeffries, 2002) observed that after alter initial communities, those return to an
78 equilibrium and converge to similar predator:prey ratio. The alternative view, the idea of a
79 historically contingent development of communities (Gleason, 1927; Diamond, 1975), suggests
80 that the effects of stochastic drift, which varies the sequence and timing of species' arrivals, can
81 cause divergences in community structure among localities, even under identical environmental
82 conditions and regional species pools (Jeffries, 1989; Drake, 1991; Fukami & Wardle, 2005).
83 These forces, although opposed, are not necessarily exclusive. Indeed, deterministic and
84 historical contingent forces may work simultaneously but their effects depend on the particular
85 site conditions and the scale of the study. In this sense, convergence has usually been detected at
86 coarse structural levels, such as functional composition, while divergence has been detected at
87 finer levels, such as taxonomic composition (Samuels & Drake, 1997). Similarly, we expected

to find functional convergence and taxonomic divergence in the macroinvertebrate community during primary succession. Thus, using both taxonomic and functional approaches help in studying the mechanisms driving succession processes. Furthermore, dispersal ability has been recognized as a central component to community development during the successional processes (Belyea & Lancaster, 1999; Young et al., 2001; Chase, 2003). Therefore,, we also expected that deterministic and historical contingency forces had different effects depending on the dispersal abilities of the organisms.

In any analysis of primary succession, extensive sampling over time is needed to adequately characterize individual ponds (Zedler & Callaway, 1999; Fairchild et al., 2000; Ruhí et al., 2012). However, studies have usually only considered the colonization phase due to their limited temporal range (Velasco et al., 1993; Ruhí et al., 2009). In an attempt to overcome this problem, an age-series approach -in which communities from a set of differently aged replicated habitats are taken to represent stages in community development from the youngest to the oldest conditions- has frequently been adopted (e.g., Barnes, 1983; Fukami & Wardle, 2005; Bloechl et al., 2010). The present study focused on macroinvertebrate organisms from isolated manmade ponds constructed at different time-periods during reclamation activities at opencast coal mines. Consequently, the studied ponds have different ages (from 1 to approx. 22 years old), were constructed under similar conditions and were affected by similar climatic conditions which made them appropriate for the use of the chronosequence approach for studying primary succession (Majer & Nichols, 1998; Walker et al., 2010). Moreover, the isolated character of the manmade ponds made them excellent places for studying the effects of the dispersal abilities of the organisms.

Specifically, the main objective of this study was to analyze changes in taxonomic and functional composition along the gradient of primary succession while taking into account the dispersal mode of the organisms. We hypothesized that organisms have different successional patterns depending on their dispersal modes. Thus, (1) the expected trend of a succession gradient from *r* to *K* strategies will be followed primarily by flightless and poorly-flying organisms and not by strong-flying organisms, and (2) although we expected that deterministic

forces mainly drove functional composition and that stochastic drift and biotic interactions mainly drove taxonomic composition, we expected to find differences when the dispersal abilities of the organisms were taken into consideration.

Materials and methods

Study site

The current study was conducted in northeastern Spain (Teruel Province), which is characterized by a continental Mediterranean climate. The manmade ponds sampled in this study (Fig. 1) were constructed during the reclamation process of several coal mines. These coal mines worked the same coal seam using similar extraction methods and followed comparable reclamation. The manmade ponds were created for the same purpose, to manage the water runoff produced in reclaimed mines to avoid contaminating natural ecosystems. We selected 19 manmade ponds covering an age range of 22 years old that were as similar as possible. Thus, the manmade ponds were integrated into endorheic basins and isolated from watercourses with the exception of two ponds, which were connected by a small stream. All the manmade ponds have water permanence, oval shapes and similar sizes (with an average of 2.5 ha). The banks of the ponds were entirely covered by *Typha* sp. (almost the only macrophyte found in the manmade ponds) with the exception of three ponds, where macrophytes are extremely scarce or absent, and aquatic vegetation was absent from most of manmade ponds. No natural ponds exist in the river basin where mines are located.

Sampling and sample processing

Two macroinvertebrate surveys were conducted; one in the spring and one in the summer of 2009. Both surveys together were thought to encompass seasonal variability because our study was focused on inter-annual, not seasonal changes in biodiversity.

The macroinvertebrate collection was restricted to shallow sites (< 1 m deep) located in the littorals of the manmade ponds. For each pond, one integrated sample from the major identified habitats (emergent plants, bottom and water column) was collected with a 250- μ m mesh hand-net using the kick-and-sweep sampling technique. Sampling was considered complete when, by visual observation, no new taxa were found. The macroinvertebrate samples were preserved in 4% formalin. In the laboratory, the samples were washed through nested sieves and the collected fauna were sorted under a stereomicroscope. From the collected fauna, insects, mollusks and annelids were identified mostly to the genus level (except for the Oligochaeta, Ceratopogonidae and Dolichopodidae, which were identified to the family level, and the Chironomidae, which were identified to the subfamily and tribe levels). Microcrustaceans (Copepoda, Cladocera, Ostracoda) were not included in this analysis.

Biological traits

To study the role of dispersal, we separately analyzed two fractions of the community, the flightless and poorly-flying organisms and strong-flying organisms, using the genus-trait information according to Tachet et al. (2000). Genus-trait information was structured using a fuzzy-coding technique (Chevenet et al., 1994): scores ranged from '0', indicating 'no affinity', to '5', indicating 'high affinity' for a given genus–trait category. Thus, in the flightless and poorly-flying category, taxa with an 'aerial active dispersal affinity' lower than 3 were included, whereas in the strong-flying category, taxa with an 'aerial active dispersal affinity' equal to or higher than 3 were included. To perform the functional characterization of the macroinvertebrate community, we selected 3 biological traits from Tachet et al., (2000): maximal body size of the last aquatic phase (with 7 categories: < 0.25, 0.25-0.5, 0.5-1, 1-2, 2-4, 4-8, > 8 cm), life cycle duration (with 2 categories: $\leq$ 1 year, > 1 year), and feeding habits (with 8 categories: absorber, deposit feeder, shredder, scraper, filter-feeder, piercer, predator, and parasite). We divided the piercer category into animal-piercers and plant-piercers according to their diet so finally 9 categories were considered in the feeding habit trait.

167 We integrated spring and summer data in the same matrix to perform the data analysis.
168 Presence/absence data were used to remove the effects of abundance and give equal weight to
169 taxa that were less common (Proctor & Grigg, 2006; Lawrence et al., 2010). Thus, working with
170 presence/absence data allows for the detection of changes in the macroinvertebrate assemblages
171 due to the appearance and disappearance of taxa instead of changes due to variations in the
172 relative abundance of taxa. This presence/absence approach is often used in long-term study of
173 macroinvertebrate trends (Lawrence et al., 2010; Arle & Wagner, 2013; Sim et al., 2013). To
174 obtain the proportion of a given trait category found in the invertebrate communities from each
175 pond, the affinity scores were weighted by the presence/absence of each genus, and these values
176 were summed for any given trait category. In addition to remove the effects of taxonomic
177 richness among the ponds, traits categories were rescaled as proportions (sum=1), such that, for
178 each trait category, values ranged from 0 (no affinity among the individuals for the trait
179 category) to 1 (all individuals of the manmade pond had exclusive affinity for the trait
180 category).

181 Moreover, we grouped the ponds into four categories according to their ages (Pond Age
182 Categories, hereafter PAC) to perform the statistical analysis: PAC1, 1-5 years old (5 ponds);
183 PAC2, 6-10 years old (5 ponds); PAC3, 11-15 years old (4 ponds); and PAC4, 16-22 years old
184 (5 ponds). In PAC 1 the pond construction was near in time, so ponds have close ages and the
185 ponds had among a minimum of 1 year old and a maximum of approximately 3 years old. In
186 each PAC there are included ponds of at least 2 studied mining areas (see Fig. 1).

187 Changes in the taxonomic and functional compositions among the PAC were explored using
188 an analysis of similarity (ANOSIM; Clarke & Green, 1988; Clarke, 1993). We performed the
189 analysis for the strong-flying organisms and flightless and poorly-flying organisms separately.
190 ANOSIM returns a test statistic R, which represents the degree of difference between the sites
191 and a p-value expressed as a percentage. When R is negative or close to 0, similarities within
192 groups (ponds from the same PAC) and among groups (ponds from different PAC) are

equivalent. In contrast, differences among the groups exist when the R values approach 1 (Clarke & Warwick, 2001). R-values > 0.5 indicated clear differences between groups with some degree of overlap (Clarke & Gorley, 2006). Taxonomic richness (n° taxa) was calculated for both strong-flying organisms and flightless and poorly-flying organisms. To test for differences among PAC in richness between dispersal abilities (strong-flying and flightless and poorly-flying), ANCOVA was performed using dispersal ability as the fixed factor, PAC as the covariable, and richness response as the variable. Moreover, to detect differences in dominance among flightless and poorly-flying organisms (proportion of flightless and poorly-flying organisms relative to the total number within each pond) among the PAC we used ANOVA. We only performed this analysis for the flightless and poorly-flying organisms because dominance of both dispersal categories is complementary. Finally, we used the similarity of percentages analysis (SIMPER; Clarke, 1993) to calculate the taxonomic and functional similarities among and within PAC. This analysis was only performed when the ANOSIM detected significant differences among the PAC. SIMPER also showed the relative contributions of individual taxa and trait categories to the similarities among groups (in this case the PAC).

To assess the convergence or divergence of taxonomic and functional composition in the communities, we calculated the similarity of ponds within each PAC using SIMPER analysis. If there was convergence, the similarity between pairs of ponds within each PAC would increase between PAC1 and PAC4. We calculated if taxonomic and functional similarity within each PAC increase (converge) or decrease (diverge) with the age applying Pearson correlations analyses.

ANOSIM and SIMPER analyses were computed with PRIMER Version 6.0 software (Clarke & Gorley, 2006). We converted taxonomic and functional data into a resemblance matrix using Bray-Curtis distances. The functional data matrix was previously square-root transformed. Because distance-based analyses such as ANOSIM and SIMPER could confound location and dispersion effects (Warton et al., 2012), we have analyzed which ones are acting in our data exploring median-variance relationship and using function “PERMDISP” option in PRIMER. Our results suggested low influence of dispersion effect in our distance-based

analyses (homogeneity of variances among PACs). Therefore, location effects (differences among PACs) and not dispersion effect (differences in PACs variability) seems to be more important for determining the results found in our study. ANCOVA and ANOVA were performed using the “aov” function from the “stats” package in R (R Core Team, 2012). Pearson correlations were calculated using "cor.test" function included in same "stats" package.

Results

Flightless and poorly-flying organisms and strong-flying organisms among the PAC

Flightless and poorly-flying organisms (40 taxa) were characterized by the Diptera, Heteroptera, Ephemeroptera, Trichoptera, Oligochaeta and Mollusca (Table 1). In contrast, strong-flying organisms (55 taxa) were primarily composed of Coleoptera, Odonata and Heteroptera, although we also found Ephemeroptera and Diptera at low proportions.

ANOSIM performed with taxonomic data showed no significant differences among the PACs, neither for flightless and poorly-flying organisms nor for strong-flying organisms (p -value > 0.05 ; Table 2). We did not find any relationship between taxonomic richness and the PACs (ANCOVA; $F_{1,34} = 1.777$, $p = 0.286$), and no differences were found in the interactions between PAC and dispersal abilities ($F_{1,34} = 2.618$, $p = 0.115$), demonstrating that the taxonomic richness of flightless and poorly-flying organisms and strong-flying organisms varied in the same way. Similarly, the dominance of flightless and poorly-flying organisms showed no significant differences among PACs (ANOVA; $F_{1,17} = 3.108$, $p = 0.0959$).

The flightless and poorly-flying organisms found in the ponds were functionally characterized as having sizes between 0.25 and 2 cm; they exhibited both short (< 1 year) and long (> 1 year) life cycle durations, although the affinity for short life cycles was slightly higher (i.e. 44% affinity for long life cycles vs. 56% affinity for short life cycles); and the community was composed of deposit feeders, scrapers, shredders, predators and animal-piercers. On the other hand, the strong-flying organisms exhibited sizes similar to the flightless and poorly-

247 flying organisms (between 0.25-0.5 cm and 1-2 cm); they showed short life cycle durations (the
248 affinity to long life cycles was really scarce, around the 1%); and they exhibited predominantly
249 predator behaviors (including animal-piercing), although they also showed high affinity towards
250 the shredders feeding habits.

251 The functional similarities among the PACs were generally high for both types of
252 organisms, ranging from 77 to 89% (Table 2). When critically analyzing the results at a
253 functional level, some weak ($R < 0.5$) but significant differences appeared among the PACs: the
254 flightless and poorly-flying organisms showed significant differences between the youngest
255 (PAC1) and oldest ponds (PAC4) while strong-flying organisms showed significant differences
256 between the youngest ponds (PAC1) and the other three PACs (Table 2). Thus, flightless and
257 poorly-flying organisms that lived in PAC1 showed higher affinities for smaller body sizes
258 ($< 0.25-0.5$ cm), shorter life cycles (< 1 year), and scraper and piercer feeding habits than did
259 PAC4 (SIMPER; Table 3). Strong-flying organisms that lived in PAC1 showed higher affinities
260 for piercer feeding habits and the largest body sizes (4-8 cm); in contrast, a higher affinity for
261 deposit feeders, scrapers and predators with variable body sizes and life cycle durations was
262 observed in the rest of the PACs (SIMPER; Tables 4, 5 and 6).

263 Taxonomic and functional convergence or divergence

264 The Pearson correlation performed with similarity data of each PAC, obtained using
265 SIMPER analysis, showed that taxonomic composition for both flightless and poorly-flying
266 organisms and strong-flying organisms significantly diverged (decreases of similarity within
267 PACs from PAC1 to PAC4; Fig. 2). However, no convergence pattern was observed for the
268 functional approaches. Instead, the functional characteristics of strong-flying organisms
269 diverged and flightless and poorly-flying organisms maintained high similarity in the four PACs
270 (Fig. 3).

272 Discussion

273 Shift from *r* to *K* strategies

274 We did not find taxonomic differences among PACs for either strong-flying organisms or
275 flightless and poorly-flying organisms. Similarly, taxonomic richness and dominance did not
276 show any significant differences among PACs regardless of dispersal abilities. At the functional
277 level, and although the similarity among PACs was quite high, some significant but small
278 differences arose. Thus, according to our hypothesis, the results suggested a slight trend from *r*
279 to *K* strategies for flightless and poorly-flying organisms along a successional gradient.
280 Specifically, the weak shift from *r* to *K* strategies detected for flightless and poorly-flying
281 organisms can be attributed to the observed increase in the organisms' affinity for larger body
282 sizes, longer life cycles and higher trophic positions (i.e., predators) along the successional
283 gradient (i.e., the PACs). The tendencies detected for body size and life cycle duration were in
284 accordance with recent studies: De Bie et al. (2012) indicated that flightless and poorly-flying
285 organisms of larger sizes need more time to arrive at new locations (in our study one of these
286 species may be *Nemotelus*), and Ruhí et al. (2012) detected an increase in the number of
287 individuals with longer life spans and later maturations over time (e.g. *Eretes* that have longer
288 life spans appeared in oldest ponds). Furthermore, the increases in predation affinity are in
289 agreement with the general tendency found in other studies concerning macroinvertebrate
290 succession in aquatic ecosystems (Schneider & Frost, 1996; Wellborn et al., 1996; Ruhí et al.,
291 2012). For flightless and poorly-flying organisms, predation is usually related to larger sizes
292 because a large body is an advantage for catching prey (Woodward & Hildrew, 2002; Brown et
293 al., 2004; Harrison & Dobson, 2008) as was the case of *Tipula* in our study. In contrast, strong-
294 flying organisms showed relatively higher affinity for larger sizes in the youngest ponds (e.g.
295 *Anax* in this study) , which is in accordance with Jenkins et al. (2007) who found that size is
296 important to the maximal dispersal distance for active dispersers. Therefore the increases in the
297 affinity for predation with pond age in strong-flying organisms could be more closely related to
298 the increase in prey abundance than to body size (Nicola et al., 2010). Indeed, the predator:prey

relationship was demonstrated to be a dynamic process through the community readjust to maintain a certain equilibrium over time (Jeffries, 2002).

Deterministic and historical contingent forces

Environmental characteristics are one of the first filters for macroinvertebrate configuration because only the organisms with functional characteristics adapted to live under the ecosystem's specific conditions are able to colonize it and establish themselves (Belyea & Lancaster, 1999; Statzner et al., 2004; Mellado-Díaz et al., 2008). Thus, because the manmade ponds were constructed under similar conditions, we expected that the communities would functionally converge if deterministic forces drove the communities (Samuels & Drake, 1997; Fukami & Wardle, 2005). The weak differences found in macroinvertebrate community among PAC suggest that, as we expected, environmental conditions may be limiting the community evolving and strongly determining the macroinvertebrate composition (Poff, 1997; Belyea & Lancaster, 1999; Statzner et al., 2004). Indeed, mining produces important ecological impacts as the aquatic pollution by heavy metals (Cravotta, 2008; Pond et al., 2008; Palmer et al., 2010) that may influence the community configuration (Clements, 1994; Merricks et al., 2007). Moreover, our results suggested that deterministic forces may have a greater effect on flightless and poorly-flying organisms because they maintained very high similarity values in functional and taxonomic characteristics beginning with the first PAC while strong-flying organisms showed a greater variability and even significantly diverged. The difference in the evolving of flightless and poorly-flying organisms and strong-flying organisms may be based on different life-history strategies (Harrison & Dobson, 2008; Verberk et al., 2008; Florencio et al., 2010). Flightless and poorly-flying organisms focus their strategy on allocate higher resources to persist in a place once they arrive (for example a high per capita invest, parental care or the formation of cocoons; Verberk et al., 2008); while strong-flying organisms focus their strategy on colonizing favorable locations which reduce the resources that can be allocated to other functions, such as fecundity (Roff, 1990; Saglam et al., 2008). The greater mobility of strong-flying organisms may involve a greater influence of random processes due a higher risk of failure as, for example,

326 locating a suitable new site (Masters et al., 2007), suffer predation *en route* (Malmqvist, 2002)
327 and fail to locate mates (Harrison & Dobson, 2008). Thus both, the lower persistence and the
328 greater mobility of strong-flying organisms, may increase the influence of historical contingent
329 forces and being, at least, partially responsible of their functional divergence (Drake, 1991;
330 Chase, 2003). Indeed, the flightless and poorly-flying organisms divergence despite maintain
331 very high functional similarity values over time support this issue. Accordingly, lower
332 predictability in community configuration was found for specialist (e.g. good flying organisms)
333 than generalist dispersing organisms (Tischendorf et al., 2003).

335 **Conclusions**

336 This study about primary succession in lentic aquatic ecosystems was performed in a unique
337 place, using ponds constructed at reclaimed opencast coal mines over the span of 22 years. As
338 we predicted, we detected a weak shift from *r* to *K* strategies for flightless and poorly-flying
339 organisms while the strong-flying organisms showed less predictable changes. Nevertheless, we
340 did not detect a clear trend in the macroinvertebrate community. This fact may be influenced by
341 the isolation character of manmade ponds, the absence of natural lentic sources of
342 macroinvertebrates and the potential hard environmental conditions of reclaimed mines.
343 Moreover the presence-absence approximation may not detect community changes exclusively
344 due to differences in organisms' abundance. On the other hand, our results showed that
345 historical contingent forces may have an important influence in the final configuration of
346 macroinvertebrate communities especially in strong-flying organisms. Therefore, our study
347 offers two interesting contributions: on the one hand, as we suggested, macroinvertebrates
348 showed different responses during primary succession depending on their dispersal abilities; on
349 the other hand, isolated ponds at reclaimed opencast coal mines may be a limiting environment
350 for the development of macroinvertebrate communities, so special attention should be done in
351 mine reclamation plans in order to improve the health of the aquatic ecosystems. The presence-
352 absence approximation allowed detecting trends related to the community evolving over time

that would be interesting to develop in future studies also using both presence-absence and abundance data.

Acknowledgements This work was supported by a research and assistance agreement between Endesa S.A and the Pyrenean Institute of Ecology-CSIC. The first author was the recipient of a Ph.D. grant from the Government of Aragón (Spain). We are grateful to the people working at the Endesa mines of Teruel and to our fieldwork assistants. Finally we would like to thank to Dr. Jeffries and the other anonymous reviewer for their helpful comments on a previous version of this manuscript.

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Table 1 Percentage contribution from each taxonomic group to the total number of taxa of flightless and poorly-flying organisms and strong-flying organisms by pond age categories (PAC).

Taxonomic group	Flightless and poorly-flying organisms				Strong-flying organisms			
	PAC1	PAC2	PAC3	PAC4	PAC1	PAC2	PAC3	PAC4
Coleoptera	0.54	0.00	0.00	0.00	24.22	26.64	20.84	16.76
Diptera	22.30	21.38	22.24	29.75	0.54	5.57	0.80	5.85
Ephemeroptera	7.32	5.86	6.81	10.85	0.67	1.63	1.20	1.06
Heteroptera	10.95	10.36	10.62	5.92	6.08	2.79	6.01	1.09
Odonata	0.00	0.00	0.00	0.00	20.26	19.67	22.44	13.09
Trichoptera	3.22	0.77	3.61	5.50	0.00	0.00	0.00	0.83
Megaloptera	0.00	0.00	0.00	1.84	0.00	0.00	0.00	0.00
Oligochaeta	2.69	3.51	3.81	4.28	0.00	0.00	0.00	0.00
Bivalva	0.00	0.00	0.00	0.55	0.00	0.00	0.00	0.00
Gastropoda	1.21	1.82	1.60	2.64	0.00	0.00	0.00	0.00

Table 2 ANOSIM and SIMPER results performed with functional data

		ANOSIM		SIMPER
		R	p-value	Similarity percentage
Flightless and poorly-flying organisms	PAC4-PAC1	0.485	0.024	89.97
Strong-flying organisms	PAC4-PAC1	0.432	0.008	77.51
	PAC3-PAC1	0.375	0.024	86.48
	PAC2-PAC1	0.300	0.048	88.57

Table 3 SIMPER performed for flightless and poorly-flying organisms. Trait category contribution (percentage) to dissimilarity between the pairs of ponds age categories (PAC; 1: 1-5 years, 2: 6-10 years, 3: 11-15 years, 4: 16-22 years) that showed significant differences in ANOSIM (see results section). Trait categories contributing globally up to 90% are shown. (LCD= Life Cycle Duration; FH: Feeding Habits; BS: Body Size).

Biological Trait	Trait category	PAC4 Average percentage	Relation	PAC1 Average percentage	Contribution (%)
LCD	>1year	0.34	>	0.24	12.93
FH	Animal-piercer	0.25	<	0.38	11.79
BS	0.25-0.5cm	0.36	<	0.48	9.56
FH	Filter-feeder	0.32	>	0.25	7.48
BS	2-4cm	0.24	>	0.17	6.39
FH	Herbivorous-piercer	0.21	<	0.29	6.37
BS	0.5-1cm	0.68	>	0.64	6.22
BS	4-8cm	0.27	>	0.22	5.83
FH	Shredder	0.45	>	0.38	5.66
FH	Predator	0.37	>	0.32	5.34
BS	≤0.25cm	0.24	<	0.25	4.48
BS	1-2cm	0.45	=	0.45	3.57
LCD	≤1year	0.94	<	0.96	3.54
FH	Scraper	0.42	<	0.43	3.08

Table 4 SIMPER performed for strong-flying organisms. Trait category contribution (percentage) to dissimilarity between PAC1 (1-5 years) and PAC2 (6-10 years). Trait categories contributing globally up to 90% are shown. (LCD= Life Cycle Duration; FH: Feeding Habits; BS: Body Size).

Biological Trait	Trait category	PAC2 Average percentage	Relation	PAC1 Average percentage	Contribution (%)
FH	Deposit-feeder	0.21	>	0.05	12.33
FH	Herbivorous-piercer	0.08	<	0.23	11.77
BS	0.5-1cm	0.38	>	0.28	10.59
BS	4-8cm	0.16	<	0.28	10.35
BS	2-4cm	0.35	>	0.31	9.03
FH	Filter-feeder	0.1	>	0.04	8.12
FH	Scraper	0.16	>	0.09	7.59
FH	Animal-piercer	0.47	<	0.54	6.26
BS	1-2cm	0.57	<	0.6	6.12
FH	Predator	0.59	>	0.56	4.86
BS	0.25-0.5cm	0.58	<	0.6	4.34

Table 5 SIMPER performed for strong-flying organisms. Trait category contribution (percentage) to dissimilarity between PAC1 (1-5 years) and PAC3 (11-16 years). Trait categories contributing globally up to 90% are shown. (LCD= Life Cycle Duration; FH: Feeding Habits; BS: Body Size).

Biological Trait	Trait category	PAC3 Average percentage	Relation	PAC1 Average percentage	Contribution (%)
BS	0.5-1cm	0.3	>	0.28	11.55
FH	Scraper	0.2	>	0.09	10.18
BS	2-4cm	0.38	>	0.31	9.28
FH	Herbivorous-piercer	0.1	<	0.23	9.1
FH	Filter-feeder	0.1	>	0.04	8.11
BS	0.25-0.5cm	0.49	>	0.6	7.6
FH	Deposit-feeder	0.1	>	0.05	6.89
BS	4-8cm	0.19	<	0.28	6.71
FH	Animal-piercer	0.45	<	0.54	6.31
FH	Predator	0.64	>	0.56	6.04
BS	1-2cm	0.64	>	0.6	5.15
LCD	≤1year	0.67	>	0.64	4.73

Table 6 SIMPER performed for strong-flying organisms. Trait category contribution (percentage) to dissimilarity between PAC1 (1-5 years) and PAC4 (16-22 years). Trait categories contributing globally up to 90% are shown. (LCD= Life Cycle Duration; FH: Feeding Habits; BS: Body Size).

Biological Trait	Trait category	PAC4 Average percentage	Relation	PAC1 Average percentage	Contribution (%)
FH	Animal-piercer	0.18	<	0.54	15.75
BS	0.25-0.5cm	0.38	<	0.6	9.66
FH	Deposit-feeder	0.27	>	0.05	8.96
FH	Herbivorous-piercer	0.04	<	0.23	7.99
FH	Scraper	0.21	>	0.09	6.78
FH	Predator	0.68	>	0.56	6.64
BS	4-8cm	0.2	<	0.28	6.55
LCD	≤1year	0.68	>	0.64	5.97
LCD	>1year	0.7	<	0.77	5.73
BS	0.5-1cm	0.4	>	0.28	5.67
BS	1-2cm	0.62	>	0.6	5.6
BS	2-4cm	0.37	>	0.31	5.34

Fig. 1 Study site and pond locations.

Fig. 2 Pond taxonomic similarity within each pond age category (PAC; 1: 0-5 years, 2: 6-10 years, 3: 11-15 years, 4: 16-22 years). A: Data for flightless and poorly-flying organisms; B: data for strong-flying organisms. Pearson correlation for similarity among PACs is shown. Poorly flying corresponded to flightless and poorly-flying organisms.

Fig. 3 Pond functional similarity within each pond age category (PAC; 1: 0-5 years, 2: 6-10 years, 3: 11-15 years, 4: 16-22 years). A: Data for flightless and poorly-flying organisms; B: data for strong-flying organisms. Pearson correlation for similarity among PACs is shown. Poorly flying corresponded to flightless and poorly-flying organisms.