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Chapter 4

Strong seasonal and macroinvertebrate community effects on nutrient mineralisation in wrack on sandy beaches

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4.1 Abstract

Sandy beaches play a significant role in nutrient cycling on the land-ocean interface, and decomposition of beach-cast macroalgae (i.e. wrack) is crucial to this connection. The decomposition and mineralisation of wrack is driven by a variety of abiotic and biotic processes, but we currently lack an understanding of how these drivers may interact. Therefore, we assessed the individual and combined effects of the macroinvertebrate community, season and drift line position on N and P mineralisation of wrack and of season and drift line position on macroinvertebrate community composition, by performing a litter bag experiment on a sandy beach. We found a strong effect of season on both macroinvertebrate community composition and N and P mineralisation. Drift line was not a driver of either macroinvertebrate community composition or N and P mineralisation, except that old drift lines have higher P mineralisation in spring while the opposite was the case in other seasons. Multiple linear regression analysis was used to test the combined effects on N and P mineralisation, showing that season (87% on average) and macroinvertebrate abundance (12% on average) together explained most of the variation in N and P mineralisation. For P mineralisation, macroinvertebrate richness and diversity also had an additional but small effect (3% and 2% on average, respectively). The independence of N and P mineralisation from drift line position indicates that wrack decay is largely uncoupled from the direct environment where decomposition occurs. Instead, the strong effects of both season and macroinvertebrate abundance on nutrient cycling suggest that nutrient hot spots may be formed on sandy beaches that accumulate wrack. Such hot spots likely support beach pioneer plant growth and enhance vegetation cover and diversity. In the light of coastal management, our study stresses the importance of leaving wrack undisturbed on sandy beaches, especially during the plant growing season.

4.2 Introduction

Coastal ecosystems provide a dynamic interface linking terrestrial and marine ecosystems (Heck et al. 2008) and perform a wide range of important ecosystem functions, such as coastal protection by buffering of wave energy and nutrient cycling (McLachlan and Brown 2006). Sandy beaches, in particular, form a unique ecosystem of their own (McLachlan 1980), where terrestrial and marine habitats strongly interact. As sandy beaches receive large amounts of exogenous organic matter that has been produced by primary and secondary producers in the ocean, they are considered to be primarily recipient ecosystems (Liebowitz et al. 2016). Ecological communities on sandy beaches depend heavily on this organic matter input for their functioning (Polis and Hurd 1996, Del Vecchio et al. 2013, Schlacher et al. 2017), especially as the internal primary production of sandy beaches is low and the ecosystem is generally bottom-up controlled (Schlacher and Hartwig 2013).

Organic material is deposited onto the beach in a drift line during retreating water. This deposition is influenced by a combination of wind, waves and currents, usually forming several semi-continuous bands of organic material parallel to the coast line. On a tide-dominated beach, multiple drift lines are formed with a shift from spring to neap tide as the water line keeps reaching lower levels on the beach, while on tide-independent beaches, a decrease in wind speed is primarily responsible for the formation of multiple drift lines by lowering sea water levels (Hammann and Zimmer 2014). Drift lines mainly consist of stranded sea grasses and sea weeds (collectively termed wrack) but may also include other organic components, such as carrion and faeces or man-made debris (Colombini and Chelazzi 2003). Young drift lines are primarily composed of freshly deposited wrack and are formed around the high water line (Orr et al. 2005). When wrack is not resuspended back into the water by high sea water levels, wrack may either remain in its current drift line or be blown further up-shore at high wind speeds, until the material becomes buried in the sand, caught by other structures (e.g. plants) or has reached a wind-dead location (Hammann and Zimmer 2014). This older, more decayed wrack is generally present in drift lines above the high water line, both higher up the beach and closer to the dune foot than young drift lines consisting of fresh wrack (Rodil et al. 2008).

Wrack that remains on the beach decomposes through a variety of abiotic and biotic processes (Colombini and Chelazzi 2003). Abiotic processes that work on deposited wrack include drying and photodegradation by the sun, erosion of organic material by wind and coverage by a layer of sand, while biotic processes include the decomposition of wrack by microbes and macroinvertebrates (Colombini and Chelazzi 2003, Orr et al. 2005). The interaction between biotic and abiotic processes may, furthermore, depend on both spatial and temporal factors, such as position across the beach (i.e. height above sea water level) and season, respectively (McLachlan and McGwynne 1989, Colombini et al. 2000, Gonçalves and Marques 2011).

Macroinvertebrates directly affect wrack decomposition by feeding on wrack, and its associated biofilm (Porri et al. 2011), thereby fragmenting the organic material (Ince et al. 2007, Salathé and Riera 2012). By decreasing wrack particle size and mixing bacteria with wrack through macroinvertebrate feeding activity, the surface area for microbial activity increases and decomposition is stimulated (Robertson and Mann 1980, Colombini and Chelazzi 2003). Also, the burrowing activities of some macroinvertebrates incorporate wrack fragments within the sand, indirectly increasing decomposition due to improvement of abiotic conditions (Inglis 1989), i.e. lower maximum temperatures and higher soil moisture content. Wrack consumption can be high and supratidal macroinvertebrates have been estimated to consume up to 80% (Griffiths and Stenton-Dozey 1981, Griffiths et al. 1983) and even 100% (Lastra et al. 2015) of the organic matter input entering the sandy beach. Therefore, the macroinvertebrate community can be a crucial driver of wrack decomposition, especially where abundances are locally high and/or consumption of wrack by individual macroinvertebrates is significant (e.g. Lastra et al. 2015, Ruiz-Delgado et al. 2016).

The interactions between macroinvertebrate community composition and wrack decomposition change over time. After deposition on the beach, wrack is swiftly colonised by macroinvertebrates, marking the start of a succession of microbes and macroinvertebrate species associated with decay stages of wrack (Colombini and Chelazzi 2003, Olabarria et al. 2007, Whitman et al. 2014, Ruiz-Delgado et al. 2016). In general, talitrids (such as the amphipod *Talitrus saltator* Montagu) and Diptera are the first colonisers of wrack, followed by insects (mainly Coleoptera) and spiders (Lavoie 1985, Ruiz-Delgado et al. 2016). With an increase in wrack age and stage of decay, changes in the macroinvertebrate abundance, richness and community composition occur (Jędrzejczak 2002b, Olabarria et al. 2007, Olabarria et al. 2010). In turn, the macroinvertebrate community affects the decomposition of wrack, mainly via consumption of organic matter (Lastra et al. 2015). When a patch of wrack has been largely decomposed, macroinvertebrates may move along to other, less decomposed wrack patches. Only species that can still feed on more decomposed wrack or use it for other purposes (e.g. habitat, egg deposition, secondary consumption) will remain (Ruiz-Delgado et al. 2016). Thus, there appears to be a tight, reciprocal interaction between the macroinvertebrate community composition and wrack decomposition (Lastra et al. 2015).

The decomposition of wrack on sandy beaches results in the release of inorganic nitrogen (N) and phosphorus (P) to the environment (i.e. mineralisation) (Dugan et al. 2011, McLachlan and Brown 2006). This may lead to a nutrient flow to the water to support marine primary production, as nutrients leach from partly decomposed wrack around the high water line (Colombini and Chelazzi 2003). This is supported by the suggestion that most eroding sandy beaches flush its nutrients, together with beach sand, back to the water, thereby returning the nutrients provided by the exogenous organic material (McLachlan and McGwynne 1989). At some conditions, nutrient hot spots may be created in the supratidal zone, locally supporting terrestrial primary (Hemminga and Nieuwenhuize 1990, Posey et al. 1999, Del Vecchio et al. 2013) and secondary production (Polis and Hurd 1996, Schlacher et al. 2017).

While the individual components related to wrack dynamics on sandy beaches are known (as discussed above), we lack an understanding on the interactions between season, macroinvertebrates and position on the beach in determining wrack decomposition and nutrient mineralisation. Identifying the main drivers of wrack decomposition and mineralisation on sandy beaches is essential to understand nutrient cycling in the sandy beach ecosystem and its connections to terrestrial and marine ecosystems. Therefore, in this study, we aimed to assess whether 1) supratidal macroinvertebrate community composition, 2) season (temporal effect), and/or 3) placement in young or old drift lines (horizontal spatial effect) are drivers of N and P mineralisation of wrack. We hypothesised that 1) a higher macroinvertebrate abundance and a more diverse community have a positive effect on N and P mineralisation of wrack, 2) N and P mineralisation of wrack is dependent on season, with a higher mineralisation in summer, and 3) N and P mineralisation of wrack is independent of drift line position. Regarding the third hypothesis; by using the same fresh wrack in both young and old drift lines, our research will allow to decouple the effects of wrack decay stage and environment on decomposition and mineralisation. To study the effect of the supratidal macroinvertebrate community, drift line and season on N and P mineralisation, we performed a litter bag experiment on a sandy beach along the Dutch coast.

4.3 Methods

4.3.1 Design of field experiment

The experiment was conducted near Scheveningen, the Netherlands (52.05 N, 4.19 E). The Netherlands is subject to a temperate climate, with a mean annual temperature of 11.3 °C and a mean annual rainfall of 891 mm recorded at Hoek van Holland, the closest weather station to the field site (Royal Netherlands Meteorological Institute (KNMI) 2015). Litter bags (for details: see below) were placed on a beach of approximately 200 m wide backed up by dunes.

To establish an environmental gradient perpendicular to the coast line, litter bags were placed either within a recently established drift line (<48 h old) directly above the high water line (HWL) or an old drift line (> several days old) higher up the beach, with at least 10 m between both lines. While the number of drift lines was greatly variable in the field, both from day to day and between seasons (personal observation), the young drift line was always the one at HWL and the old drift line was higher up on the beach and the one closest to the dune foot. Young drift lines consisted of freshly deposited wrack which was generally still moist from the sea water, while old drift lines consisted of dry wrack that had been positioned on the beach longer ago and was (partly) buried by sand.

The experiment was performed in three seasons to study seasonal effects on N and P mineralisation: spring (May 2015), summer (August 2015) and autumn (October 2015). Litter bags consisted of freshly collected litter of *Ulva lactuca* Linnaeus, a common green macroalgae species found among wrack on Dutch and other European sandy beaches (e.g. Barreiro et al. 2011). Litter bags were incubated in the drift lines for approximately two weeks, after which they were harvested. This period was chosen as it was expected that *U. lactuca* would have been decomposed for more than 50% (Buchsbaum et al. 1991, Mews et al. 2006), which would allow wrack to be significantly decomposed and differences in wrack decay to have emerged. The fast decomposition was considered a major advantage of *U. lactuca* over e.g. the more slowly decomposing macroalgae *Fucus vesiculosus* Linnaeus which is more common among wrack (Barreiro et al. 2011), as a shorter incubation period made the experiment less vulnerable to disturbances. Thus, there were two treatments within this litter bag experiment: drift line position (young or old) and season (spring, summer or autumn). As it was expected that litter bags would be lost at the beach due to both natural (e.g. storms) and anthropogenic (e.g. digging up of litter bags by humans or dogs) disturbances, we placed 30 litter bags for each combination of treatments. This resulted in 60 litter bags placed on the beach per season and 180 litter bags in total for the entire experiment.

Prior to placement of a litter bag in the drift line, *U. lactuca* was rewetted by submerging the litter bag in sea water at the site for 5 seconds. This was done to mimic the moisture content of natural wrack when freshly deposited on the beach and to have similar initial moisture conditions in each litter bag. Litter bag margins were buried up to 3 cm deep, securing the edges under the sand but allowing the middle part of the bag (± 10 cm^2) to be uncovered. In each drift line, we marked ten positions that were each 10 m apart, resulting in 90 m of drift line parallel to the coast to place litter bags. At each position, three litter bags were placed together in a triangle no more than 50 cm from the drift line. To prevent removal of litter bags due to disturbance, a 23-cm iron pin was placed 30 cm deep in the sand at the center of each cluster of three litter bags, to which each of the litter bags was attached with a piece of sisal twine. All twines were buried in the sand. GPS coordinates were taken at each cluster of three litter bags for retrieval purposes. At the end of the entire experiment, 95 of the 180 litter bags could be retrieved, however, and 64 litter bags had enough wrack material (≥1 g of wrack with remaining sand) left for chemical analysis.

4.3.2 Litter bag preparation

We had access to a *U. lactuca* culture which provided for a steady supply of sea weed throughout the year. For each season, a fresh batch of *U. lactuca* of approximately 5-6 kg wet weight was collected from the cultures at The Netherlands Institute for Sea Research (NIOZ), at Texel, the Netherlands, 7-10 days before the start of the experiment. *Ulva lactuca* was air-dried by hanging it on plastic lines at 25 °C for at least 72 hours. From this air-dried material, 6 subsamples were dried in the oven at 70 °C for 72 hours to determine the relationship between air-dry and oven-dry *U. lactuca*, which was used to estimate the oven-dry weight added at the start of the experiment. Litter bags were made 15 x 20 cm in size and constructed of white nylon mesh, with a smaller mesh size on the lower (1 mm) than the upper (10 mm) side of the litter bag. This was done to prevent fragmented sea weed of falling through larger openings upon harvest, but to allow larger macroinvertebrates to enter the litter bag, respectively. After cleaning air-dried sea weed from dead animals and other organic contaminations with a pair of tweezers, 4.0 ± 0.1 g air-dry *U. lactuca* was added to each litter bag and sealed on the edges with hot glue.

4.3.3 Macroinvertebrate collection

At harvest, each retrieved litter bag was detached from the iron pin and gently placed in an empty plastic 1-L pot, avoiding animals to escape. Below each litter bag, a 20 x 20 x 15 cm volume of sand was collected using a stainless-steel rectangle corer with a small spade and sieved over a 10-mm sieve directly in the sea. Remaining animals were collected and stored in 70% ethanol. On the same day of harvest, material was transported to the laboratory and intact litter bags with the remaining wrack were placed in a Tullgren extractor for at least one week, to extract all macroinvertebrates (van Straalen and Reijninks 1979). Care was taken to transfer only a limited amount of sand together with the litter bag into the Tullgren funnel to prevent clogging of the vials. Animals were collected in a vial with 70% ethanol, counted and identified to the lowest possible taxonomic level (Table A4.1). Air-dried litter bags with remaining wrack were stored in 1-L plastic pots until further processing the week after.

4.3.4 Measurements on macroalgae

Remaining wrack was removed from the litter bags and gently washed with artificial sea water (30‰ salt content; Instant Ocean, Aquarium Systems, Inc., Mentor, OH, USA) to remove sand and organic contaminants attached to the wrack. Wrack samples were dried at 70 °C for 72 hours, weighed for dry biomass (including remaining sand) and ground into a fine powder in a ball mill (MM400, Retsch, Haan, Germany) and homogenised by mixing the ground sample with a steel stirrer. Each sample was split into two: one part was used to determine ash free dry weight and the other part was used for chemical analysis (total C, N and P content). Three *U. lactuca* samples taken at t=0 for each season were also analysed for total C, N and P content and ash free dry weight. Total C and N content of wrack were determined by weighing 8-12 mg (3-4 mg for *U. lactuca* at t=0) of ground sample in a tin cup, followed by dry combustion with a Flash EA1112 elemental analyser (Thermo Scientific, Rodana, Italy). For total P content, a 50 mg (100 mg for *U. lactuca* at t=0) subsample was digested in 1 ml of a 1:4 mixture of 37% (by volume) HCl and 65% (by volume) HNO_3, in a closed Teflon cylinder for 6 h at 140 °C.

Samples were then diluted with 4 ml demineralised water and total P content was measured colorimetrically (Murphy and Riley 1962). Ash free dry weight was determined by combusting a homogenised subsample of 20 mg (10 mg for *U. lactuca* at t=0) for 5 h at 550 °C. All fresh and dry masses were determined up to the nearest 0.001 g.

4.3.5 Data and statistical analysis

Of the 64 retrieved litter bags, four litter bags that did not contain any macroinvertebrates were excluded, resulting in 60 litter bags to be used in the data analysis. N and P mineralisation (in mg per g initial wrack dry weight) was calculated as the absolute difference in total N or P content of wrack at harvest and at the start of the experiment per litter bag, which was divided by the initial dry weight of wrack for standardisation. The total N and P content in wrack at the start of the experiment was estimated using % N and % P of t=0 wrack material from each respective season. Macroinvertebrate abundance ($\log^{10}$-transformed), richness (number of taxa) and Shannon's diversity index (H') were also calculated per litter bag.

Prior to analysis, all data were tested for homogeneity of variances (Levene's test) and normal distribution (Shapiro-Wilk test). When these assumptions were not met, a log (N mineralisation and macroinvertebrate abundance) or square root (macroinvertebrate richness and diversity) transformation was performed on the original data. First, we analysed whether macroinvertebrate community metrics were significantly affected by drift line (two levels: young and old) and season (three levels: spring, summer and autumn) by running two-way ANOVAs. Secondly, we analysed how N and P mineralisation were affected by drift line (two levels: young and old) and season (three levels: spring, summer and autumn) in two-way ANOVAs. Two-way ANOVAs were followed by Tukey post hoc tests where applicable. Finally, macroinvertebrate abundance, richness or diversity were added to drift line and season in a multiple regression analysis to determine whether macroinvertebrate community metrics added predictive power to explaining variance of N and P mineralisation. Only the interaction between drift line and season was included in each multiple regression model, following the results of the two-way ANOVAs. The relative importance of each of the factors in the multiple regression model was calculated by decomposing R^2 into non-negative contributions that sum to the total of R^2, taking care of the dependency of ordering within the regression model by averaging over all possible orderings of regressors, following Grömping (2006). All statistical analyses were done in R, version 3.2.3 (R Core Team 2015).

4.4 Results

4.4.1 Macroinvertebrate abundance, richness and diversity across drift line and season

Macroinvertebrate abundance, richness and diversity did not differ between drift lines, but did significantly differ between seasons (Table 4.1). In autumn, macroinvertebrate abundance was higher than in spring and summer (190 ± 130 ind. against 26 ± 45 and 75 ± 75 ind. litter bag^{-1}, respectively; Tukey's post hoc, t=5.4, p<0.001 and t=2.8, p=0.02, respectively; Figure

Table 4.1. Results of the two-way ANOVAs for macroinvertebrate abundance, richness and Shannon's diversity with drift line and season as factors. An asterix indicates a significant p-value (<0.05), while an open circle indicates a trend (*p*<0.10).

	df	F	*P*
Macroinvertebrate abundance			
Drift line	1	0.5	0.50
Season	2	18.1	<0.001*
Drift line * Season	2	4.9	0.01*
Macroinvertebrate richness			
Drift line	1	0.6	0.44
Season	2	18.0	<0.001*
Drift line * Season	2	0.4	0.66
Macroinvertebrate diversity			
Drift line	1	3.5	0.07 °
Season	2	50.5	<0.001*
Drift line * Season	2	1.5	0.23

Table 4.2. Results of the two-way ANOVAs for N and P mineralisation with drift line and season as factors. An asterix indicates a significant p-value (<0.05).

	df	F	*P*
N mineralisation			
Drift line	1	0.1	0.73
Season	2	59.0	<0.001*
Drift line * Season	2	0.7	0.50
P mineralisation			
Drift line	1	0.07	0.79
Season	2	227.7	<0.001*
Drift line * Season	2	5.4	<0.01*

4.1). In summer, macroinvertebrate richness was higher than in spring and autumn (3.2 ± 1.2 taxa against 1.5 ± 1.1 and 1.4 ± 0.7 taxa litter bag^{-1} respectively; Tukey's post hoc, t=4.8, *p*<0.001 and t=-5.8, *p*<0.001, respectively; Figure 4.1). Macroinvertebrate diversity was higher in summer than in spring and autumn (H' = 0.8 ± 0.2 against 0.2 ± 0.3 and 0.02 ± 0.04, respectively; Tukey's post hoc, t=4.6, *p*<0.001 and t=-6.8, *p*<0.001, respectively; Figure 4.1). In addition, macroinvertebrate diversity was significantly different between spring and autumn (Tukey's post hoc, t=-2.4, *p*<0.05), with a higher macroinvertebrate diversity in spring. Only for macroinvertebrate abundance there was a significant interaction effect between drift line and season (Table 4.1). In summer, the macroinvertebrate abundance was higher in old than in young drift lines (127 ± 77 ind. against 28 ± 32 ind. litter bag^{-1}; Tukey's post hoc, t=3.3, *p*=0.02), while macroinvertebrate abundance in spring and autumn was similar between young and old drift lines (Tukey's post hoc, t=0.2, *p*=1.0 and t=-2.3, *p*=0.2 respectively).

Table 4.3. Results of multiple linear regression analysis for the relationship between N and P mineralisation and macroinvertebrate community (implemented as either abundance (log-transformed), richness or Shannon's diversity), drift line and season. N and P mineralisation were the dependent variables in these models (Y in the model Y ~ X). In each model, the interaction between drift line and season was included. An asterix indicates a significant p-value (<0.05), while an open circle indicates a trend (*p*<0.10).

			df	F	*p*	% of model explained
N mineralisation						
Abundance	Overall fit: R^2=0.63, *p* <0.001*	Abundance	1	29.4	<0.001*	11
		Drift line	1	0.5	0.47	<1
		Season	2	37.6	<0.001*	88
		Drift line * Season	2	0.7	0.49	1.2
Richness	Overall fit: R^2=0.63, *p* <0.001*	Richness	1	3.3	0.07 °	1.2
		Drift line	1	0.1	0.82	<1
		Season	2	50.1	<0.001*	98
		Drift line * Season	2	0.4	0.65	<1
Diversity	Overall fit: R^2=0.63, *p* <0.001*	Diversity	1	3.5	0.07 °	1.2
		Drift line	1	0.0	0.97	<1
		Season	2	50.0	<0.001*	98
		Drift line * Season	2	0.4	0.66	<1
P mineralisation						
Abundance	Overall fit: R^2=0.89, *p* <0.001*	Abundance	1	168.6	<0.001*	13
		Drift line	1	0.5	0.48	<1
		Season	2	159.1	<0.001*	85
		Drift line * Season	2	5.0	<0.01*	2.0
Richness	Overall fit: R^2=0.89, *p* <0.001*	Richness	1	7.8	<0.01*	2.5
		Drift line	1	0.0	0.93	<1
		Season	2	237.7	<0.001*	94
		Drift line * Season	2	4.7	0.01*	1.9
Diversity	Overall fit: R^2=0.89, *p* <0.001*	Diversity	1	21.9	<0.001*	1.7
		Drift line	1	0.5	0.47	<1
		Season	2	237.6	<0.001*	96
		Drift line * Season	2	5.6	<0.01*	2.1

4.4.2 N and P mineralisation across drift line and season

For N mineralisation, there was a significant effect of season only (Table 4.2). In spring, N mineralisation was lower (9.36 ± 0.89 mg g initial wrack^{-1}) than in summer and autumn (14.78 ± 2.60 and 15.05 ± 2.57 mg g initial wrack^{-1}, respectively; Tukey's post hoc, t=8.0, *p*<0.001 and t=8.7, *p*<0.001, respectively; Figure 4.2). Also for P mineralisation, only the main effect of season was significant (Table 4.2). In spring, P mineralisation was lowest (1.14 ± 0.07 mg g initial wrack^{-1}) while P mineralisation was highest in autumn (1.57 ± 0.07 mg g initial wrack^{-1}), with an intermediate P mineralisation in summer (1.30 ± 0.07 mg g initial wrack^{-1}; Figure 4.2). In contrast to N mineralisation, there was a significant interaction effect of drift line and season for P mineralisation (Table 4.2). P mineralisation was slightly higher in old drift lines in spring (1.17 ± 0.06 against 1.10 ± 0.06 mg g initial wrack^{-1}), while the opposite was the case for summer and autumn with a slightly higher P mineralisation in young drift lines (1.33 ± 0.07 against 1.27 ± 0.08 mg g initial wrack^{-1} and 1.59 ± 0.07 against 1.55 ± 0.06 mg g initial wrack^{-1}, respectively; Figure 4.2).

4.4.3 Combined effect of the macroinvertebrate community, drift line and season on mineralisation

Within the multiple linear regression models, the effects of drift line and season on N and P mineralisation were maintained, with a significant positive effect of season on both N and P mineralisation and only on P mineralisation a significant positive effect of drift line in combination with season (Table 4.3). In addition, macroinvertebrate abundance showed a significant positive effect on N mineralisation, while richness and diversity did not significantly affect N mineralisation. For P mineralisation, macroinvertebrate abundance, richness and diversity all had a significant positive impact (Table 4.3). Season explained 85 to 98% of the fitted model, while macroinvertebrate abundance, richness and diversity explained 11 to 13%, 1 to 3% and 1 to 2% respectively. Adding interaction terms between macroinvertebrate abundance, richness or diversity and drift line and season respectively, did not change these patterns (see Appendix, Table A4.2 and A4.3).

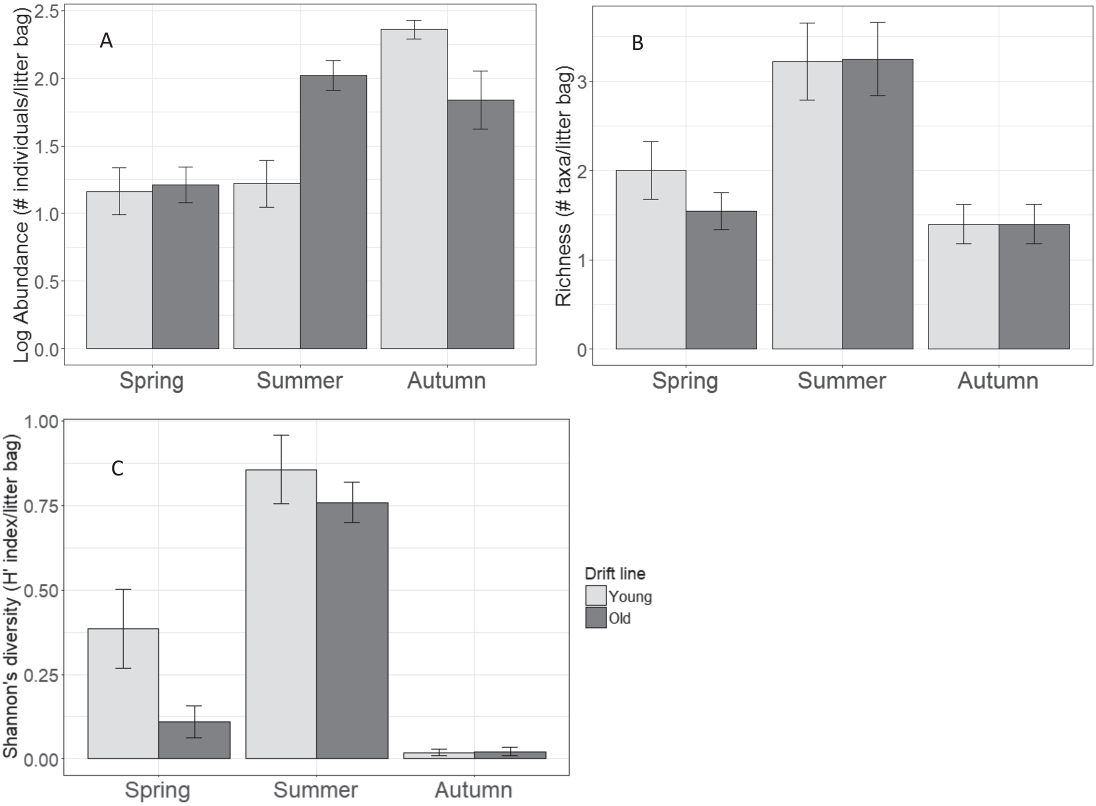

Figure 4.1. Means for macroinvertebrate A) abundance, B) richness and C) Shannon's diversity per drift line and season. Error bars indicate the standard error from the mean.

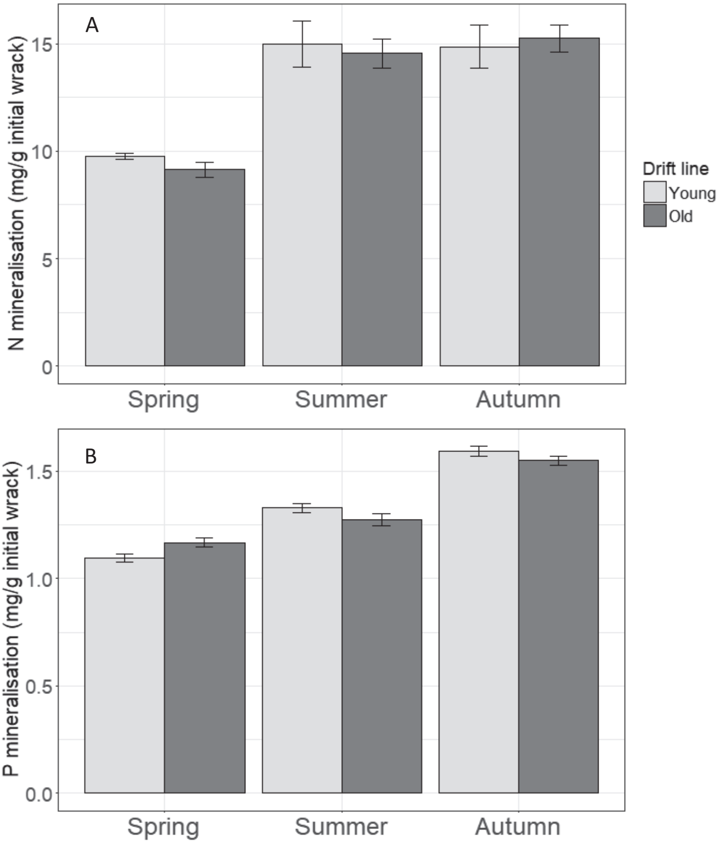

Figure 4.2. Means for A) N mineralisation and B) P mineralisation of wrack per drift line and season. Error bars indicate the standard error from the mean.

4.5 Discussion

In this study we aimed to assess the individual and combined effects of the supratidal macroinvertebrate community, drift line position and season on N and P mineralisation of wrack. We found a strong effect of season on both macroinvertebrate community composition and N and P mineralisation. Season explained most of the variation in N and P mineralisation. Drift line position only showed an effect on macroinvertebrate abundance and P mineralisation in interaction with season. When adding macroinvertebrate abundance to the multiple regression model, the total explained variance remained the same, while both season and macroinvertebrate abundance affected N and P mineralisation, indicating that a part of the seasonal dynamics in N and P mineralisation was explained by macroinvertebrate abundance. Moreover, P mineralisation was also explained by macroinvertebrate richness and diversity, but to a lesser extent than by macroinvertebrate abundance and season. Together, this indicates that both season and macroinvertebrate community metrics, in particular abundance, played a crucial role in nutrient cycling on our sandy beach.

4.5.1 Macroinvertebrate abundance is a strong driver of wrack mineralisation

As to our first hypothesis, we did find that macroinvertebrate abundance had a strong positive effect on both N and P mineralisation of wrack, but macroinvertebrate community richness and diversity were only for P mineralisation a significant explanatory variable (see also Appendix, Figure A4.1 and A4.2). A higher macroinvertebrate abundance may enhance wrack decomposition and mineralisation through an increase in feeding, shredding and digging activities performed by these macroinvertebrates, and the subsequent positive effects on microbial activity (Inglis 1989, Jędrzejczak 2002b, Ince et al. 2007, Lastra et al. 2008, Salathé and Riera 2012). Macroinvertebrate abundance was higher in autumn than in spring and summer, which was mainly due to the presence of large numbers of Diptera larvae (mainly *Fucellia* sp. and *Coelops* sp., see Appendix, Table A4.1). Abundance of Diptera larvae on wrack typically peaks between one to two weeks of field incubation (Jędrzejczak 2002b), which coincides with the litter bag harvest after two weeks in this study. Abundances of the terrestrial amphipod *Talitrus saltator* were close to zero in this study, as *T. saltator* is an early succession species and its abundance on wrack peaks around four days (Jędrzejczak 2002b) up to seven days (Olabarria et al. 2007) after wrack is deposited on the beach. As we sampled the wrack after two weeks, we did not capture this species and our results reflect the cumulative effects across multiple macroinvertebrate community successional stages. Hence, we were unable to disentangle whether an early peak in *T. saltator* abundance or the later peak of Diptera larvae was responsible for part of the N and P mineralisation of wrack. As both groups potentially have a strong effect on wrack decomposition (e.g. Stenton-Dozey and Griffiths 1980, Lastra et al. 2008, Salathé and Riera 2012, Lastra et al. 2015), the relative importance of individual supratidal macroinvertebrate species on wrack mineralisation thus requires further study.

For P mineralisation, macroinvertebrate richness and diversity were additionally identified as significant predictors, although with a lower magnitude than macroinvertebrate abundance. This may be related to a greater variety in nutritional requirements among macroinvertebrates when more or different macroinvertebrate species are present on wrack, potentially influencing wrack mineralisation. As a clear macroinvertebrate succession occurs on wrack (Olabarria et al. 2007, Whitman et al. 2014, Ruiz-Delgado et al. 2016), early-succession macroinvertebrate species, such as *T. saltator*, may indirectly facilitate late succession macroinvertebrate species, e.g. due to fragmentation of wrack to smaller particle size as a result of their feeding activities on wrack and its associated biofilm (e.g. Olabarria et al. 2007). Late-succession macroinvertebrate species may then be able to feed on wrack that was previously not available to them in terms of particle size or nutritional quality (Heard 1994). This mechanism could also operate within a specific wrack successional stage, where large-sized macroinvertebrates may facilitate small-sized macroinvertebrates, as a greater variety in invertebrate body size is associated with higher mineralisation rates (Heemsbergen et al. 2004, Handa et al. 2014). Finally, niche-partitioning may occur among co-occurring macroinvertebrate species that are dependent on the same food source but have different temporal and spatial uses (Colombini et al. 2000, Jaramillo et al. 2003, Lastra et al. 2010, Bessa et al. 2014a). As a result, at a greater variety of supratidal macroinvertebrate species, more wrack is utilised, resulting in a higher wrack mineralisation. It nevertheless remains unclear why this appears to hold for P mineralisation only. This difference between N and P mineralisation might be due to a different distribution of nitrogen- and phosphorus-rich compounds within wrack: if organic compounds with a high P content have been made available from wrack due to a richer and more diverse macroinvertebrate community, this might have resulted in a higher P mineralisation without affecting N mineralisation.

Importantly, there appears to be a critical, reciprocal relationship between the macroinvertebrate community and wrack mineralisation. Macroinvertebrate community composition associated with wrack has been indicated to influence wrack decomposition and mineralisation (Dugan et al. 2003, Lastra et al. 2008, Urban-Malinga et al. 2008, this study). In turn, factors such as wrack biomass, structure and nutritional quality all affect macroinvertebrate community composition (Colombini and Chelazzi 2003, Dugan et al. 2003, Olabarria et al. 2007). These processes occur simultaneously in time and space, posing the future research challenge to fully disentangle the relative importance of the reciprocal processes occurring between wrack mineralisation and macroinvertebrate community composition, both at multiple temporal and spatial scales.

4.5.2 Season is a strong driver of wrack mineralisation

In accordance with our second hypothesis, N and P mineralisation of wrack was highly dependent on season. N mineralisation was high in both summer and autumn, while P mineralisation was highest in autumn. Seasonal variation in mineralisation may be related to seasonal differences in weather conditions, such as temperature and moisture content. Moderately high temperatures increase wrack decay rates on sandy beaches by stimulating feeding rates of macroinvertebrates and microbial activity (Colombini and Chelazzi 2003, Lastra et al. 2015). At the same time, wrack has temperature insulating properties that help maintain a more stable, moderate temperature within the patch of wrack compared to the overlaying air temperature (Coupland et al. 2007). A high precipitation coupled to low evaporation, and increased moisture conditions e.g. due to burial by sand, stimulates microbial activity and results in releasing more nutrients from wrack via decay (Lavery et al. 2013, Coupland et al. 2007). During our study, both the mean temperature (18.6 °C) and mean precipitation (131.3 mm) were highest in August (summer) compared to May (spring; 12.1 °C and 50.6 mm, respectively) and October (autumn; 10.4 °C and 34.5 mm, respectively) (Royal Netherlands Meteorological Institute (KNMI) 2015). The higher temperatures in summer may have resulted in enhanced wrack N mineralisation, assuming that moisture was not limiting. While the net impacts of increased temperature (causing higher evaporation) and increased precipitation on the moisture content of wrack in the field are not known, it appears likely that an additional driver was responsible for the high wrack mineralisation observed in autumn. As indicated above, macroinvertebrate abundance was strongly related to N and P mineralisation, and the highest macroinvertebrate abundance was observed in autumn. This suggests that a part of the seasonal dynamics in N and P mineralisation may be explained by macroinvertebrate abundance.

In addition to seasonal effects related to differences in weather conditions and macroinvertebrate abundances, the initial quality of wrack used in the experiment may be responsible for the strong effect of season on N and P mineralisation of wrack. The C/N ratio of the wrack used varied between seasons. When adding the C/N ratio of wrack to the multiple regression models, C/N ratio was a strong predictor of N and P mineralisation in addition to season (for P mineralisation only) and macroinvertebrate community metrics (see Appendix, Table A4.4). Indeed, the N content of wrack is one of the potential drivers of wrack decay on sandy beaches (Jędrzejczak 2002a). In conclusion, N and P mineralisation of wrack was strongly determined by season, which was due to both seasonal differences in weather conditions and the initial wrack quality used.

Jędrzejczak (2002a) proposes that the order of importance for factors affecting wrack decay rate are type of wrack (i.e. macroalgae species), temperature, internal N content of wrack and decomposer activity. Similarly, in terrestrial ecosystems, temperature and plant growth form composition are identified as most important drivers of decomposition, with a smaller role for litter quality within species (e.g. Cornelissen et al. 2007). In addition, climate is considered to be a dominant driver of decomposition over macrodetritivores (García-Palacios et al. 2013), which may be even more pronounced on sandy beaches as they are classically viewed as being dominated by the environment (McLachlan and Brown 2006). Our results indicate that season was the main driver of N and P mineralisation followed by macroinvertebrate abundance, supporting the above hypothesis on the order of importance of factors influencing mineralisation of wrack.

4.5.3 Wrack mineralisation is largely independent of drift line position

Finally, we hypothesised that N and P mineralisation of wrack was independent of drift line horizontal spatial position, which is what we generally observed in this study. Only for P mineralisation there was an additional interaction effect between drift line and season, indicating P mineralisation was higher in old drift lines in spring, while the opposite was the case for summer and autumn with a higher P mineralisation in young drift lines. This interaction effect may be explained by variation in the distance of the chosen drift lines to the high water in this study across seasons, which was dependent on stochastic variation in drift line formation within each season. Again, it then remains unclear why N and P mineralisation showed a different response. This cannot be related to the macroinvertebrate community: even though macroinvertebrate abundance showed an interaction between drift line and season, the pattern for macroinvertebrate abundance was dissimilar from that of P mineralisation.

By using the same fresh wrack in both young and old drift lines, our research allowed to decouple the effects of wrack decay stage and environment on decomposition and mineralisation. Previous studies focussed on naturally decomposing wrack across the sandy beach, where young and old wrack were directly compared with each other. For example, Dugan et al. (2011) found that N and P concentrations in beach sand differed with position across the beach, with the highest concentration of nutrients observed around the high water line where fresh wrack accumulated. Until now it was, however, unclear whether this was due to wrack decay stage or environmental conditions. Even so, differences in wrack carbon and nutrient dynamics are commonly related to environmental conditions: there is a strong environmental gradient across the entire sandy beach, combining both the intertidal and supratidal zone (McLachlan and Jaramillo 1995, Wood and Bjorndal 2000), where the higher supratidal zone is relatively stable in terms of the abiotic environment compared to the intertidal zone. Wrack in older drift lines higher up the beach has consistently higher temperatures and lower moisture contents than fresh wrack in young drift lines around the high water line (Ruiz-Delgado et al. 2015). Moreover, wrack (and its associated macroinvertebrate community) close to the dune foot is less prone to rewetting by tidal inundation, redistribution across the beach by waves and wind, and inundation of macroinvertebrate colonisers (Wood and Bjorndal 2000, Defeo and Gómez 2005, Coupland et al. 2007). Despite these apparent environmental differences, our results suggest that instead differences in wrack decay stage are the dominant driver of differences in wrack decomposition between drift lines. Macroinvertebrate abundance was similar between drift lines, possibly because species are mobile, allowing them to easily migrate between wrack patches deposited across the sandy beach (Colombini et al. 2000). It thus seems that the stable macroinvertebrate community and a high degree of migration of supratidal macroinvertebrate species across the supratidal zone of the sandy beach may have caused wrack mineralisation to be largely independent from drift line position.

4.5.4 Implications for coastal management

In light of coastal management, our study stresses the importance of leaving wrack undisturbed on sandy beaches, especially in summer. In summer, wrack mineralisation was moderate to high, macroinvertebrate abundance was intermediate and macroinvertebrate richness and diversity were both high. However, summer is also the season with a high recreation pressure on sandy beaches (Kelly 2016). Recreation both has a strong direct and indirect impact on wrack and its associated macroinvertebrate community (e.g. Schlacher and Thompson 2012, McLachlan et al. 2013). Therefore, an integrated approach for coastal management of sandy beaches is necessary to facilitate wrack mineralisation and support the sandy beach ecosystem, by mitigating anthropogenic disturbance to wrack and its macroinvertebrate communities on sandy beaches as much as possible.

4.5.5 Conclusion

On sandy beaches that accumulate wrack, both season and the macroinvertebrate community, especially its abundance, were found to play a crucial role in nutrient cycling. This may result in nutrient hot spots on the sandy beach, supporting beach pioneer plant growth and enhanced vegetation cover and diversity (Del Vecchio et al. 2013, Del Vecchio et al. 2017). Our study also highlights that wrack decomposition and mineralisation on sandy beaches is a complex process, with many interacting drivers. The fate of wrack and its reciprocal relation with the macroinvertebrate community composition in particular, therefore requires to be studied in more detail to support the sandy beach ecosystem and its management as a whole.

4.6 Appendix

4.6.1 Macroinvertebrate species and counts

Litter bags filled with *Ulva lactuca* Linnaeus were harvested after two weeks of incubation on a sandy beach and upon inspection, we found 18 macroinvertebrate taxa and 5777 macroinvertebrate individuals in total (Table A4.1). The two most common taxonomic groups belonged to the Diptera, where *Fucellia* sp. larvae and *Coelops* sp. larvae were most abundant (4624 and 814 individuals, respectively), formed the largest part of the total number of individuals (80.04 and 14.09 %, respectively) and they were widely present among the total number of litter bags (81.97 and 39.34 %, respectively; Table A4.1).

Table A4.1. Macroinvertebrate species and their total number of individuals, percentage of the total number of individuals and their presence across litter bags, for all litter bags combined.

Taxonomic group	Total number of individuals	% of total number of individuals	Present in x % of the litter bags
Crustacea			
Talitrus saltator (Talitridae)	2	0.03	3.28
Coleoptera			
Dyschirius sp. (Carabidae)	18	0.31	14.75
Bledius sp. (Staphylinidae)	18	0.31	16.39
Staphylinidae	5	0.09	8.20
Scarabaeidae (most likely *Aegialia arenaria*)	1	0.02	1.64
Coleoptera adult	2	0.03	3.28
Coleoptera larvae	2	0.03	3.28
Diptera			
Fucellia sp. larvae (Anthomyiidae)	4624	80.04	81.97
Coelops sp. larvae (Coelopidae)	814	14.09	39.34
Lonchaeidae larvae	3	0.05	1.64
Diptera larvae undetermined 1	82	1.42	9.84
Diptera larvae undetermined 2	186	3.22	11.48
Scatophagidae	3	0.05	3.28
Canacidae	3	0.05	4.92
Chironomidae	10	0.17	14.75
Bibionidae	1	0.02	1.64
Hemiptera			
Aphididae	2	0.03	3.28
Hymenoptera			
Formicidae (most likely *Lasius psammophilus)*	1	0.02	1.64
All taxa (18 taxa in total)	5777		

4.6.2 Full multiple regression model

A multiple regression model to test the effect of drift line, season and macroinvertebrate community metrics (abundance, richness and diversity) on N and P mineralisation of wrack was performed. When including all interactions within the multiple regression models, the same patterns were observed as when only the interaction effect of season and drift line was included (Table 4.3), except for an additional significant positive effect on P mineralisation by the interaction between abundance and drift line in the model including abundance (Table A4.2 and A4.3).

4.6.3 Dissecting the effect of season

Large differences were recorded in C/N ratio between seasons (data not shown), but it was unclear whether season affected the C/N ratio of wrack at harvest via seasonal weather conditions or differences in initial wrack quality. When adding the C/N ratio of wrack at harvest to the multiple regression model, the C/N ratio was significantly related in each model to N and P mineralisation (Table A4.4). Season, however, only significantly affected P

Table A4.2. Results of multiple regression analysis for the relationship between N mineralisation and macroinvertebrate community (implemented as either abundance (log-transformed), richness or Shannon's diversity), drift line and season. N mineralisation was the dependent variables in these models (Y in the model Y ~ X). In each model, all interactions were included. An asterix indicates a significant p-value (<0.05).

			df	F	*p*
Abundance	Overall fit: R^2=0.57, *p* <0.001 *	Abundance	1	27.1	<0.001 *
		Drift line	1	0.4	0.51
		Season	2	29.7	<0.001*
		Abundance * Drift line	1	0.3	0.60
		Abundance * Season	2	0.2	0.86
		Drift line * Season	2	0.5	0.63
		Abundance * Drift line * Season	2	0.4	0.69
Richness	Overall fit: R^2=0.58, *p* <0.001 *	Richness	1	2.2	0.14
		Drift line	1	0.0	0.84
		Season	2	43.4	<0.001*
		Richness * Drift line	1	1.2	0.28
		Richness * Season	2	0.4	0.70
		Drift line * Season	2	0.3	0.74
		Richness * Drift line * Season	2	0.7	0.48
Diversity	Overall fit: R^2=0.57, *p* <0.001 *	Diversity	1	1.8	0.19
		Drift line	1	0.0	0.94
		Season	2	42.7	<0.001*
		Diversity * Drift line	1	0.4	0.52
		Diversity * Season	2	0.5	0.62
		Drift line * Season	2	0.3	0.77
		Diversity * Drift line * Season	2	0.5	0.60

Table A4.3. Results of multiple regression analysis for the relationship between P mineralisation and macroinvertebrate community (implemented as either abundance (log-transformed), richness or Shannon's diversity), drift line and season. P mineralisation was the dependent variables in these models (Y in the model Y ~ X). In each model, all interactions were included. An asterix indicates a significant p-value (<0.05).

			df	F	*p*
Abundance	Overall fit: R^2=0.89, *p* <0.001 *	Abundance	1	171.0	<0.001 *
		Drift line	1	0.4	0.56
		Season	2	146.9	<0.001*
		Abundance * Drift line	1	4.8	0.03 *
		Abundance * Season	2	1.5	0.24
		Drift line * Season	2	4.9	0.01 *
		Abundance * Drift line * Season	2	0.1	0.89
Richness	Overall fit: R^2=0.88, *p* <0.001 *	Richness	1	13.6	<0.001 *
		Drift line	1	0.0	0.96
		Season	2	202.9	<0.001*
		Richness * Drift line	1	1.6	0.22
		Richness * Season	2	0.0	0.97
		Drift line * Season	2	4.7	0.01 *
		Richness * Drift line * Season	2	0.3	0.72
Diversity	Overall fit: R^2=0.88, *p* <0.001 *	Diversity	1	37.4	<0.001 *
		Drift line	1	0.8	0.38
		Season	2	199.2	<0.001*
		Diversity * Drift line	1	1.2	0.28
		Diversity * Season	2	0.7	0.49
		Drift line * Season	2	5.5	<0.01 *
		Diversity * Drift line * Season	2	0.2	0.82

mineralisation and with a much smaller magnitude than C/N ratio (Table A4.4). This suggests that the strong effect of season (in the models without the C/N ratio, Table 4.3), originates from both seasonal weather conditions and initial differences in wrack quality.

Table A4.4. Results of multiple regression analysis for the relationship between N and P mineralisation and macroinvertebrate community (implemented as either abundance (log-transformed), richness or Shannon's diversity), C/N ratio, drift line and season. N and P mineralisation were the dependent variables in these models (Y in the model Y ~ X). In each model, the interaction between drift line and season was included. An asterix indicates a significant p-value (<0.05), while an open circle indicates a trend (*p*<0.10).

			df	F	*p*
N mineralisation					
Abundance	Overall fit: R^2=0.60, *p* <0.001*	Abundance	1	28.9	<0.001 *
		C/N ratio	1	59.3	<0.001 *
		Drift line	1	0.6	0.46
		Season	2	2.3	0.11
		Drift line * Season	2	0.5	0.58
Richness	Overall fit: R^2=0.59, *p* <0.001*	Richness	1	2.3	0.14
		C/N ratio	1	83.3	<0.001 *
		Drift line	1	0.5	0.48
		Season	2	2.9	0.06 °
		Drift line * Season	2	0.3	0.76
Diversity	Overall fit: R^2=0.59, *p* <0.001*	Diversity	1	1.9	0.18
		C/N ratio	1	83.7	<0.001*
		Drift line	1	0.6	0.45
		Season	2	2.9	0.06 °
		Drift line * Season	2	0.3	0.77
P mineralisation					
Abundance	Overall fit: R^2=0.89, *p* <0.001*	Abundance	1	167.5	<0.001 *
		C/N ratio	1	156.0	<0.001 *
		Drift line	1	0.5	0.49
		Season	2	67.9	<0.001 *
		Drift line * Season	2	4.1	0.02 *
Richness	Overall fit: R^2=0.89, *p* <0.001*	Richness	1	14.5	<0.001 *
		C/N ratio	1	326.1	<0.001 *
		Drift line	1	1.1	0.29
		Season	2	54.3	<0.001 *
		Drift line * Season	2	4.1	0.02 *
Diversity	Overall fit: R^2=0.89, *p* <0.001*	Diversity	1	39.2	<0.001 *
		C/N ratio	1	346.9	<0.001 *
		Drift line	1	5.3	0.02 *
		Season	2	35.1	<0.001 *
		Drift line * Season	2	4.7	0.01 *

4.6.4 Scatter plots of macroinvertebrate community metrics against N and P mineralisation

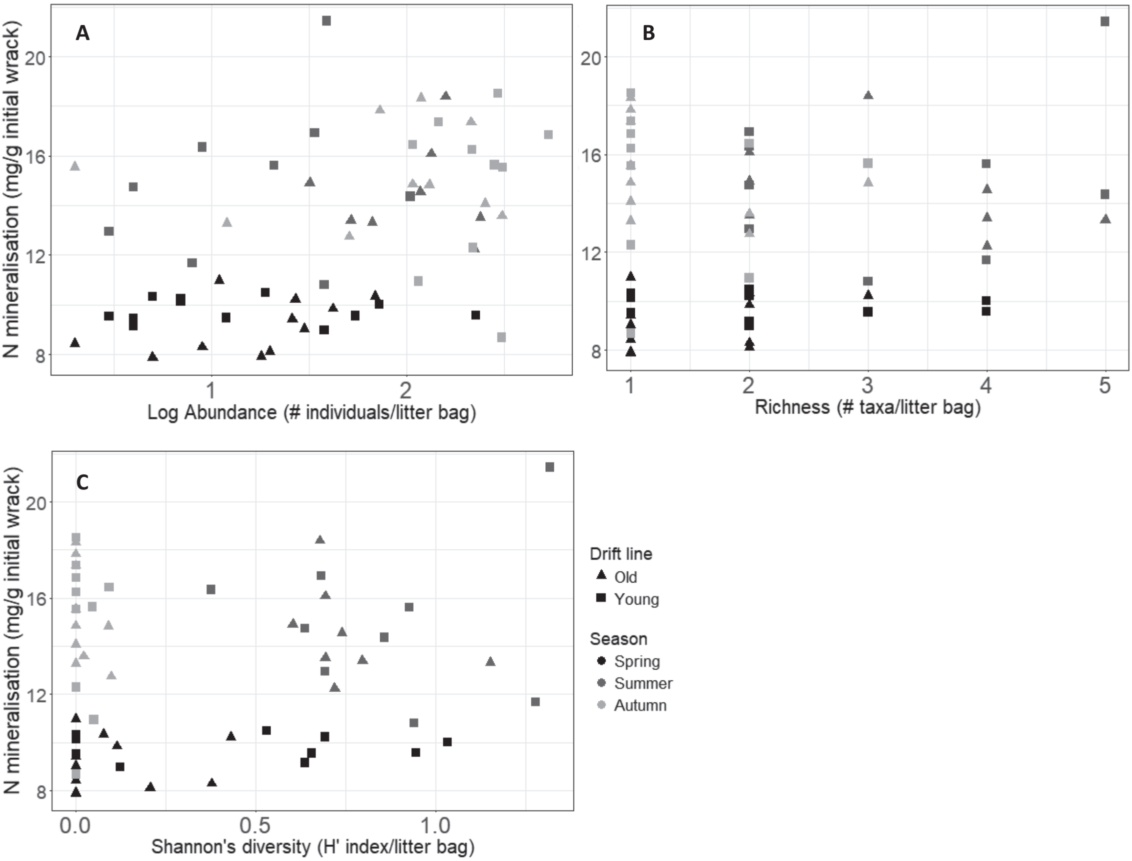

Figure A4.1. Scatterplot of A) abundance, B) richness and C) Shannon's diversity against N mineralisation. Shapes of points indicate drift line and colours of points indicate season. Each point represents one litter bag.

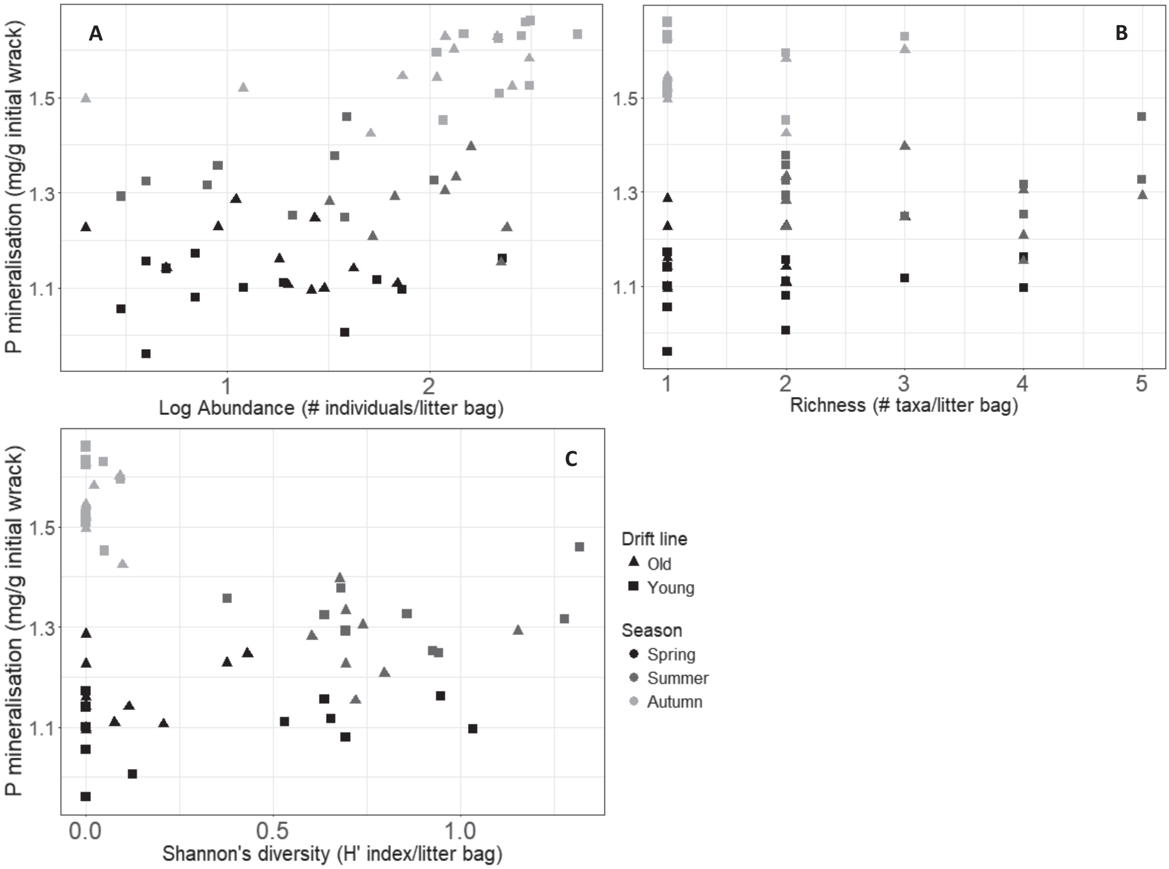

Figure A4.2. Scatterplot of A) abundance, B) richness and C) Shannon's diversity against P mineralisation. Shapes of points indicate drift line and colours of points indicate season. Each point represents one litter bag.