Living on the edge

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2018

document version
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Chapter 5

Growth of pioneer beach plants is strongly driven by buried macroalgal wrack, while macroinvertebrates affected plant nutrient dynamics

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This chapter has been submitted
for publication

5.1 Abstract

Sandy beaches depend heavily upon marine organic input, such as macroalgae, as internal organic matter productivity is low. The fate, however, of this marine organic material after being deposited onto the beach (termed wrack) and its impact on pioneer vegetation needs to be further elucidated. In particular, the effect of various drivers of wrack decomposition and nutrient mineralisation on pioneer vegetation growth at sandy beaches are largely unknown. We studied the effect of wrack burial and macroinvertebrate presence on decomposition-driven nutrient availability and beach pioneer plant growth. To this end, we performed a mesocosm experiment manipulating *Fucus vesiculosus* wrack access to the supratidal amphipod *Talitrus saltator*, and used *Cakile maritima* and *Elytrigia juncea* as phytometers to estimate wrack-derived nutrient supply. Buried wrack had a strong positive effect (2-3 times greater) on plant mass, N and P content of *C. maritima* compared to surface wrack, while effects on *E. juncea* were largely absent. In addition, macroinvertebrate-facilitated decomposition was important for increasing nutrient availability, but this did not result in an increase in plant growth. We conclude that the burial of wrack by a thin layer of sand is a crucial driver of beach pioneer plant growth primarily due to an increase in sediment moisture content. This supports the importance of management practices that allow deposited wrack to remain and be buried on the sandy beach for a long period of time, which will have positive effects on beach pioneer plant growth and possibly embryo dune formation.

5.2 Introduction

Ecosystems are open entities, thus adjacent ecosystems typically exchange organic matter and nutrients between them (Polis et al. 1997, Marcarelli et al. 2011). In low productive ecosystems, such as sandy beaches, exogenous dead organic matter and nutrients are transferred through detritus in the receiving ecosystem (Polis and Hurd 1996). In addition, ecosystem boundaries are continually crossed by organisms, where biologically possible (Nakano and Murakami 2001, Barrett et al. 2005). Coastal ecosystems are unique in the sense that they provide an interface, linking terrestrial and marine ecosystems across the world (Heck et al. 2008). These ecosystems perform a wide range of important ecosystem functions, such as nutrient cycling and the buffering of wave energy (McLachlan and Brown 2006). In particular on sandy beaches, which form a unique ecosystem of its own (McLachlan 1980), terrestrial and marine habitats heavily interact.

Sandy beaches receive large amounts of marine exogenous organic matter that has been produced by primary and secondary producers, and are therefore considered to be primarily recipient ecosystems (Liebowitz et al. 2016). Sea grasses and sea weeds are important primary producers that grow attached to a (rocky) substratum, but become detached as a result of severe hydrodynamic conditions and are consequently deposited onto the beach (Suursaar et al. 2014). Drift lines mainly contain stranded sea grasses and sea weeds (collectively termed wrack), but may also include other organic components, such as carrion or faeces (Colombini and Chelazzi 2003). Wrack supply to the beach is highly variable in time and space and is driven by, among others, the buoyancy capacity of the wrack, hydrodynamic forces and beach type

and geomorphology (Orr et al. 2005). Moreover, the location of wrack on the beach, i.e. the distance to the mean sea level isocline, is strongly determined by tidal amplitudes which change monthly to annually (e.g. Plag and Tsimplis 1999) resulting in several drift lines parallel to the water line. The initial quality of freshly deposited wrack depends on the identity of the sea grass or sea weed species and their anatomical, physiological and chemical traits at the moment of detachment and transportation (Oldham et al. 2014), as well as on the relative contribution of carrion in the wrack. This finally results in a spatiotemporal diverse drift line composition at the sandy beach. As the internal primary production of sandy beaches is very low and the ecosystem is generally bottom-up controlled (Schlacher and Hartwig 2013), the input and subsequent pathway of wrack-bound energy and nutrients is crucial to the functioning of the sandy beaches. Therefore, understanding the fate of marine-derived organic matter that enters the sandy beach is key for understanding its ecosystem functioning.

Once wrack is stranded on the beach, this material decays and decomposes through a variety of abiotic and biotic processes (Colombini and Chelazzi 2003). Abiotic processes that work on freshly deposited wrack resulting in its degradation include photodegradation, erosion by wind-blown sand and coverage of wrack by a layer of sand. Over time, wrack in drift lines higher up the beach generally becomes buried by sand through aeolian transportation and other natural processes, where it locally enhances organic matter content and changes the physical structure of the sand, which is especially relevant in sandy beaches (Rossi and Underwood 2002). The aging and burial of wrack change the microclimate and habitat properties for microbial decomposers and macroinvertebrates, making wrack an ephemeral but stabilised habitat in terms of temperature and moisture content for supratidal macroinvertebrates (Ince et al. 2007, Ruiz-Delgado et al. 2015). An important biotic process influencing wrack decomposition is the swift colonisation of wrack by microbes and invertebrates, after which a succession of species starts (Olabarria et al. 2007). As the volume and quality of wrack alter over time, changes in macroinvertebrate abundance, richness and community composition occur (Jędrzejczak 2002b, Olabarria et al. 2007). Macroinvertebrates that feed on wrack, and its associated biofilm (Porri et al. 2011), fragment the organic material (Salathé and Riera 2012). By decreasing wrack particle size and mixing of bacteria and fungi with organic matter through feeding, 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, stimulating an increase in decomposition, due to enhanced activity of decomposers by more favourable abiotic conditions (Inglis 1989). All these (a)biotic processes potentially affect the decomposition of wrack and the release of organically-bound nutrients, but how these are interacting remains largely unknown. Specifically, the relationship between natural sand burial of wrack and macroinvertebrate-facilitated decomposition has not been previously studied.

During and after the processing of organic material by detritivores, nutrients may flow back to the sea to support marine primary production (McLachlan 1980, Dugan et al. 2011) or create nutrient hot spots and locally support terrestrial primary (Hemminga and Nieuwenhuize 1990, Del Vecchio et al. 2013) and secondary production (Polis and Hurd 1996, Schlacher et al. 2017). As sandy beach communities are considered to depend more heavily

on marine subsidies than vice versa (Liebowitz et al. 2016), our focus in this study was on the role of macroinvertebrate-facilitated decomposition of wrack on terrestrial plant growth. The supratidal zone provides adverse conditions for most plant species due to among others high salinity, low moisture content and low nutrient availability of the sandy sediment (Pakeman and Lee 1991a). Wrack patches, however, are a unique micro-habitat for beach pioneer plants and an important nutrient and habitat source for animals within sandy beaches (Williams and Feagin 2010, Del Vecchio et al. 2013). Indeed, beach pioneer plants benefit in terms of growth from a pulse in nutrient availability, especially nitrogen (Pakeman and Lee 1991b). However, the role of abiotic (burial by sand) and biotic (macroinvertebrate activity) factors, influencing wrack decomposition and nutrient availability, for beach pioneer plant growth needs to be further experimentally tested.

Hence, the aims of this study were to test the effects of 1) wrack burial, 2) macroinvertebrate presence and 3) their interaction on decomposition-driven nutrient supply and beach pioneer plant growth. We hypothesised that 1) buried wrack would have a more positive effect on decomposition-driven nutrient supply and beach pioneer plant growth than wrack on the surface, as buried wrack has a higher moisture content and can more easily be decomposed. We further hypothesised that 2) macroinvertebrate presence would have a positive effect on decomposition-driven nutrient supply and beach pioneer plant growth. The common terrestrial amphipod *Talitrus saltator* (Monatagu, 1808) used in this experiment was expected to play an important role in decomposition by feeding on and thereby fragmenting the wrack and by burrowing wrack fragments. As the wrack decomposes, more nutrients become available in the sand below the wrack. This in turn is expected to enhance pioneer beach plant growth as nutrient-limitation for beach plant growth is removed when growing in a drift line. Finally, we hypothesised 3) there is an interaction effect between wrack burial and macroinvertebrate presence on decomposition-driven nutrient supply and beach pioneer plant growth. We expected that wrack on the surface would be less palatable to *T. saltator* as it holds less moisture (see Ruiz-Delgado et al. 2015) and a lower microbial biomass, hence food, and thus negatively impacts the facilitation by macroinvertebrates of decomposition and subsequent beach pioneer plant growth.

5.3 Methods

To test the effect of macroinvertebrate presence and wrack burial on wrack decomposition and beach pioneer plant growth, we conducted a climate room experiment for which we assembled mesocosms consisting of pots of sand with different combinations of wrack burial, macroinvertebrate presence and plant species.

5.3.1 Wrack collection and preparation

We used the brown sea weed *Fucus vesiculosus* as wrack, as it is a major component of wrack found upon Dutch sandy beaches (personal observation; see also Nienhuis 1970). It is a preferential food source for the supratidal amphipod *Talitrus saltator* (Lastra et al. 2008), which is used in this experiment as macroinvertebrate species (see below). Fresh *F. vesiculosus* was collected from the rocky pier near IJmuiden, the Netherlands (52.46 N, 4.55 E) during low tide, two weeks prior to the experiment. Sea weeds were cut loose with a pair of scissors at

the stipe, ± 2 cm above the holdfast and stored in a plastic bucket. Directly after collection, *F. vesiculosus* was spread out on tables in the laboratory to dry at room temperature (20 °C) for three days and stored in a dry place until the start of the experiment.

5.3.2 Animal collection

The litter-feeding terrestrial amphipod *Talitrus saltator* is a common inhabitant of wrack on Dutch sandy beaches (see e.g. Van Colen et al. 2006). We collected *T. saltator* individuals from the beach near Scheveningen, the Netherlands (52.05 N, 4.19 E) not more than two weeks before the start of the experiment. Animals were collected from bare sand in the intertidal zone with visible holes (entrance to burrows of *T. saltator*) and from fresh wrack patches deposited during receding tide. Sand was sieved over a 1 mm sieve and remaining animals were collected with a pair of soft tweezers. Fresh wrack was gently shaken above a 1 mm sieve to stir the animals and those fallen onto the sieve were collected with a pair of soft tweezers. Animals were transported to the laboratory in 1L plastic pots with a 10 cm layer of moist sand from the collection site. Upon arrival in the laboratory the same day, collected animals were transferred to a terrarium filled with a 8-10 cm thick layer of quartz sand (Multiquartz, Lelystad, the Netherlands). Sand had a 0.25-0.40 mm grain size and was free from organic matter by burning. The terrarium was finally covered with mesh (<0.5 mm mesh size) and placed in a climate room (12:12 h light/dark regime, 20:15 °C day/night temperature regime and an 85:75 % RH day/night air humidity). Freshly-cut *F. vesiculosus* sea weed was added *ad libitum* as a food source and ± 20 mL demi-water was sprayed every other day to keep the environment moist. The last 24 h before the start of the experiment all sea weed was removed to starve the animals and to empty their guts.

5.3.3 Plant cultivation

We used two common pioneer plant species of Dutch sandy beaches: *Cakile maritima* and *Elytrigia juncea* (see e.g. Speybroeck et al. 2008a, Doing 1995). *Cakile maritima* is an annual forb that typically grows on buried wrack lines (Davy et al. 2006), while *E. juncea* is a perennial grass that can be found at the dune foot and initiates embryo dune formation by capturing sand, or within stabilised stands of *C. maritima* (Speybroeck et al. 2008a, Davy et al. 2006). *Cakile maritima* seeds were collected by hand at the beach near Scheveningen, the Netherlands (52.05 N, 4.19 E) in November 2014 and December 2015, after which they were stored cool (5 °C) and dark upon arrival in the laboratory until sowing commenced. We grew *C. maritima* plants from these seeds five weeks before the start of the experiment. Seeds were removed from their fruits and, after soaking for 2 hours in demi-water, five seeds were randomly placed in a Petri-dish lined with filter paper moistened with demi-water (5-10 mL) until saturated. Petri-dishes were placed in a climate room (see above for conditions) and covered with plastic trays to allow germination in the dark. Every two days we gently transferred seedlings with a pair of soft tweezers to small pots (diameter 8 cm, height 7 cm) filled with a 6-cm layer of quartz sand (same sand as used as above), placed on a saucer (diameter 12.5 cm, height 2 cm) to catch remaining water added to the pot. Seedlings were watered once a week with 50 mL half-strength Hoagland solution (3mM KNO_3, 2 mM $Ca(NO_3)$, 1 mM $NH_4H_2PO_4$, 0.5 mM $MgSO_4$, 25 µM H_3BO_3, 1 µM KCl, 2 µM $MnSO_4$, 2 µM $ZnSO_4$, 0.1 µM

$CuSO_4$, 0.1 µM $(NH_4)_6Mo_7O_{24}$ and 10 µM Fe(Na)EDTA) and once a week with 50-100 mL demi-water, depending on the size of the plants, until the start of the experiment.

As we were insufficiently successful in growing *E. juncea* seedlings from root stocks collected in the field, we collected mature *E. juncea* plants (with a shoot length of at least 20 cm) instead and used these directly in the experiment. Approximately 100 *E. juncea* plants were collected 7 days before the start of the experiment on the beach 'De Hors', Texel, the Netherlands (53.00 N, 4.74 E) and were, upon arrival in the laboratory, placed in a plastic bucket (diameter 34 cm, height 26 cm) with a layer of 30 cm quartz sand (same sand as used as above) in the climate room. On the day of collection, 500 mL half-strength Hoagland solution was evenly added to the bucket containing all *E. juncea* plants. Sand was kept moist by adding 500 mL demi-water every other day until the start of the experiment. As we collected more *E. juncea* plants than necessary, we randomly selected 47 individuals to be used in the experiment.

5.3.4 Experimental design

We used a 2-way design to study the effect of wrack burial (surface or buried) and macroinvertebrates (present or absent) on the growth of two individual beach pioneer plant species (*C. maritima* and *E. juncea*). For each treatment we had eight replicates, which were divided over eight blocks (Table 5.1). In addition, we performed a nutrient addition test to determine if a potential positive effect of wrack addition on plant growth could be related to nutrient limitation. For this purpose, we used three treatments for both *C. maritima* and *E. juncea*: nutrient addition, inoculum addition and no nutrient addition (plant only). An inoculum addition treatment was included to account for the effect of indirectly adding extra nutrients in treatments that received wrack and inoculum. For each of these three treatments we had five replicates, which were divided over five blocks (Table 5.1). In a climate room, all mesocosms were placed within combined blocks (eight in total). This finally resulted in five blocks with fourteen mesocosms (combining main treatments and the nutrient addition test treatments) and three blocks with eight mesocosms (remaining mesocosms of main treatments). Blocks and mesocosms within blocks were moved around randomly twice a week. In total, there were 94 mesocosms and each mesocosm was randomly assigned to one of the above treatments.

Each mesocosm (plastic pot, diameter 14 cm; height: 14 cm) was filled with a 9 cm layer of quartz sand (same sand as used as above). Each mesocosm assigned to a wrack treatment received 14 g of dry *F. vesiculosus*, which corresponds to a potential nutrient addition (if all nutrients would be released from wrack) of 0.37 g N and 0.05 g P (data not shown). In treatments with surface wrack, this resulted in a 2.5-3 cm thick layer of loosely packed wrack placed on top of the sand, covering the entire surface of the mesocosm. In treatments that contained buried wrack, this resulted in a layer of 2.5-3 cm loosely packed wrack placed on top of the sand and covered with approximately 150 g sand so that the top of the wrack was just visible, with only tiny parts of wrack peeking out at the sand surface (Figure 5.1). Before placing wrack in the mesocosm, 50 mL of wrack inoculum (see below) was added onto the wrack to account for wrack-associated decomposer microorganisms and as food source for macrodetritivores which may have died-off while air-drying. For those treatments with macroinvertebrates present, we randomly added eight individuals of *T. saltator* (± 0.3 g total

fresh biomass or 52 individuals m^{-2}) corresponding to field densities encountered in wrack (19 ± 24 individuals m^{-2} (Bessa et al. 2014b); 91 ± 18 individuals m^{-2} (Ruiz-Delgado et al. 2016)). Females with brood sacks were omitted from the mesocosms. Animals were placed on top of the sand, after which most of them started burying themselves into the sand. Each mesocosm contained either one *C. maritima* or *E. juncea* plant. All mesocosms were covered with a nylon mesh (mesh size 0.5 mm) secured by tape on the pot edges, with a hole (square of 1 cm^2) to allow the stem of the plant to pass while keeping the animals in the mesocosms. A piece of white nylon wool (Pond Filter Wool, VT, Velda Group, the Netherlands) was wrapped around the stem to protect it from the sharp edges of the plastic mesh, and to prevent animals from escaping. All mesocosms were placed in a climate room with a 12:12 h light/dark regime (light intensity: 500 µmol m^{-2} s^{-1}; lamp type: Philips CDM-TMW 315 W/942), a 20:15 °C day/night temperature regime and an 85:75 % RH day/night air humidity. These circumstances correspond to late spring/early summer conditions in the Netherlands. The bottom of the mesocosms was lined with tightly woven plastic root canvas, which allowed water and solutes to pass but kept all other material inside the mesocosm. All mesocosms were placed on a saucer to collect leaching water after watering mesocosms.

5.3.5 Additions prior to and during the experiment

The day before the start of the experiment, each mesocosm received 250 mL demi-water and 50 mL 50 mM NaCl to ensure equal starting conditions in moisture and salt content. A low salt content has a positive effect on beach pioneer plant growth (Lee and Ignaciuk 1985, Debez et al. 2004). Wrack inoculum was prepared by adding 3.7 kg of freshly collected *Fucus vesiculosus* wrack to 3.5 L demi-water. After 3 hours of soaking, the inoculum was strained to remove larger wrack particles (>1mm). All main treatments and the inoculum treatment of the nutrient addition treatments were given 200 mL demi-water and 50 mL inoculum at the start of the experiment, while the nutrient addition treatment without inoculum was given 250 mL demi-water. All mesocosms received 125 mL demi-water twice a week during the experiment. The nutrient addition treatments that received nutrient solution instead were watered twice a week with 125 mL nutrient solution that contained 0.03 g N (0.43 mL of 5 M NH_4Cl stock solution) and 0.004 g P (0.13 mL of 1 M NaH_2PO_4 stock solution). In total, plants in these treatments received 0.24 g N and 0.03 g P, which is in the same range as the amount of N and P available in the amount of wrack added in this experiment. The experiment was run for four weeks in June-July 2016.

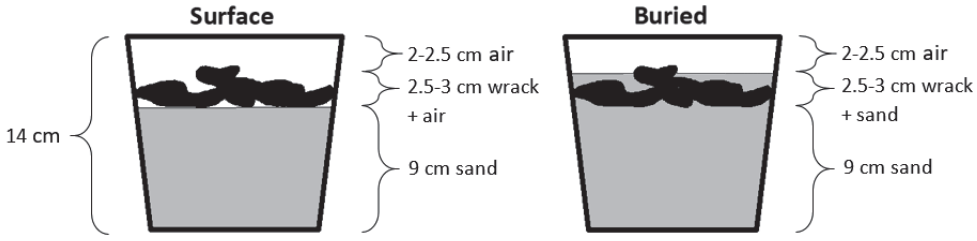

Figure 5.1. Graphical representation of the wrack burial treatments in the main experiment, indicating the depth and material of the layers.

Table 5.1. Summary of the experimental design indicating the mesocosms included for both testing the main treatments and the nutrient addition test. Within brackets it is indicated how many mesocosms were included in the subset excluding mesocosms where no animals were retrieved upon harvest.

Main treatments	Wrack burial	Macroinvertebrates	Plant species	*n*	Total *n*
	Surface	Present	*C. maritima*	8 (6)	
			E. juncea	8 (6)	
		Absent	*C. maritima*	8 (8)	
			E. juncea	8 (8)	
	Deep	Present	*C. maritima*	8 (3)	
			E. juncea	8 (3)	
		Absent	*C. maritima*	8 (8)	
			E. juncea	8 (8)	
					64 (50)
Nutrient addition test	Addition		Plant species	*n*	Total *n*
	Nutrients		*C. maritima*	5	
			E. juncea	5	
	Inoculum		*C. maritima*	5	
			E. juncea	5	
	None		*C. maritima*	5	
	(control)		*E. juncea*	5	
					30

5.3.6 Measured variables

For a subset of specimens (n=10), we collected the fresh and dry mass (to the nearest 0.001 g) at t=0 for the roots, shoot (*E. juncea* only), stem (*C. maritima* only) and leaves (*C. maritima* only). We determined the fresh mass of the total plant for all individual plants at the start of the experiment and we measured fresh weight of wrack for all mesocosms. Upon harvest, we collected the fresh and dry mass of the roots, shoot (*E. juncea* only), stem (*C. maritima* only), flowering heads (*C. maritima* only), green leaves (*C. maritima* only) and (shedded) brown leaves (*C. maritima* only). In addition, we determined the fresh and dry mass of remaining wrack and the numbers of *T. saltator* found dead or alive. For wrack, the moisture content (%) at harvest was calculated. *Talitrus saltator* individuals were considered dead either when we found the body of a non-moving individual or did not retrieve the body at all upon harvest, which were taken together to calculate the presumed mortality. Total shoot mass of *C. maritima* consisted of the stem, green and brown leaves and flowering head combined. This distinction was not made for *E. juncea* plants, which were all in the vegetative stage and without entire leaves browning. Upon harvest, samples were oven-dried for 72 h at 70 °C. Dried samples were ground into a fine powder in a ball mill (MM400, Retsch, Haan, Germany) and homogenised by mixing the ground sample with a steel stirrer. Total N concentration of plant parts were determined by dry combustion with a Flash EA1112 elemental analyser (Thermo Scientific, Rodana, Italy). For P content, a 50 mg 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). To correct for sand trapped between plant roots, a homogenised root subsample was combusted at 550 °C to obtain mass loss on ignition. Total N concentration was used to determine the total N content of the plants which can be considered as a measure of total N uptake. Finally, the N/P ratio based on dry mass upon harvest was calculated to have a measure for the type of nutrient limitation in the plant and its organs (see Appendix for results on the N/P ratio).

5.3.7 Data and statistical analysis

First, we analysed the mortality of *T. saltator* individuals to determine whether observed mortality was related to treatments. To test the effect of both wrack (two levels: surface or buried) and plant species (two levels: *C. maritima* and *E. juncea*) on mortality, a two-way ANOVA was performed. Mortality was higher in buried (90 ± 16%) than in shallow (70 ± 28%) wrack treatments (ANOVA, df = 1, F = 6.1, p = 0.02). No differences in mortality were found between plant species (ANOVA, df = 1, F = 1.6, p = 0.21) and there were no interactions between wrack burial and plant species (ANOVA, df = 1, F = 1.2, p = 0.29). Given the large number of non-retrieved individuals and the unknown contribution of them to the processes measured (while individuals found dead will at least have contributed to wrack decomposition and mineralisation for part of the experimental period), we decided to include only those mesocosms in which macroinvertebrates were retrieved upon harvest, either dead or alive, in further analysis. Exclusion of mesocosms without retrieved animals upon harvest resulted in 18 mesocosms remaining for the macroinvertebrate treatment (n=6 for shallow buried wrack and n=3 for deep buried wrack, for each of the two plant species, see Table 5.1).

We chose to present the absolute final mass, nutrient content and N/P ratio of the different plant organs as plants grew large quickly: the relative increase of total plant biomass was on average 317% (± 278%) and 70% (± 101%) for *C. maritima* and *E. juncea,* respectively. Biomass at t=0 was therefore considered small relative to the biomass increase and focussing on the absolute final biomass simplifies both the analysis and interpretation of the results. 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 (main treatments: dry mass (total shoot), P content (total plant, total shoot and roots), N/P ratio (total plant, total shoot and roots) of *C. maritima*; dry mass (total plant, total shoot and roots), N content (total plant, total shoot and roots), P (total shoot), N/P ratio (total plant and roots) of *E. juncea*; nutrient addition treatments: N content (total shoot) of *E. juncea*), a log transformation was performed on the original data. To test the effect of both wrack (two levels: surface or buried) and macroinvertebrates (two levels: present or absent) on each of the variables plant mass, N and P content and N/P ratio, a two-way ANOVA was performed for each plant organ (total plant, total shoot and roots) and for each plant species (*C. maritima* and *E. juncea*), separately. We performed a Kruskal-Wallis test to test the effect of macroinvertebrates (two levels: presence or absence) and plant species (two levels: *C. maritima* and *E. juncea*) on wrack moisture percentage separately. To evaluate the nutrient addition treatments (three levels: nutrient addition, inoculum addition and plant only) on each of the variables plant dry mass,

N and P content and N/P ratio, we performed a one-way ANOVA for each plant organ (total plant, total shoot and roots) and for each species, separately. The results of the nutrient addition treatments can be found in the Appendix. ANOVAs were followed up with Tukey's post hoc tests if relevant. All statistical analyses were done in R, version 3.2.3 (R Core Team 2015).

5.4 Results

5.4.1 Wrack moisture content

Surface-facing wrack had a lower moisture content than buried wrack (61.3 ± 6.4 % and 81.7 ± 3.3 %, respectively; Kruskal-Wallis test, df = 1, $\chi^2 = 47.3$, $p < 0.001$). There were no significant differences in wrack moisture content between plant species (Kruskal-Wallis test, df = 1, $\chi^2 = 0.27$, $p = 0.60$) or macroinvertebrate treatments (Kruskal-Wallis test, df = 1, $\chi^2 < 0.1$, $p = 0.98$).

5.4.2 Plant biomass

For *Cakile maritima*, wrack burial resulted in a strong and significant increase in the dry mass of the total plant, total shoot and roots (Table 5.2, Figure 5.2). Dry mass was 3.4 ± 1.2 g against 1.7 ± 0.5 g for the total plant, 2.9 ± 1.0 g against 1.5 ± 0.4 g for the total shoot and 0.6 ± 0.2 g against 0.2 ± 0.2 g for the roots, for buried and surface wrack, respectively. Thus, plant dry mass was approximately two times as high when wrack was buried as opposed to surface wrack. No significant effects were found for macroinvertebrate presence on the dry mass of the total plant, total shoot or roots. For *Elytrigia juncea*, there were no significant differences of either wrack burial or macroinvertebrate presence on the dry mass of either the total plant, total shoot or roots (Table 5.2, Figure 5.2).

5.4.3 Plant nitrogen content

The overall pattern for N content, for both plant species and all plant organs, was highly similar to the pattern of dry mass (Table 5.3, Figure 5.3), except that for *C. maritima* there was a significant negative effect of macroinvertebrate presence on the N content of the total plant. This similarity suggests that the biomass increase of *C. maritima* in the buried wrack treatment was strongly coupled to increased N availability in the soil. When macroinvertebrates were present, the N content of the total plant was lower than when macroinvertebrates were absent, both for buried and surface wrack (buried: 64.2 ± 22.5 mg N against 72.7 ± 12.6 mg N, surface: 24.4 ± 7.5 mg N against 25.2 ± 8.2 mg N, respectively). Moreover, when wrack was buried or placed on the surface, N content of *C. maritima* was 39.3 ± 9.1 mg N against 14.0 ± 4.2 mg N for the total shoot and 19.5 ± 5.6 mg N against 5.7 ± 3.5 mg N for the roots, respectively. Thus, the N content was approximately three times as high when wrack was buried as opposed to surface wrack.

5.4.4 Plant phosphorus content

The overall pattern for P content, for both plant species and all plant organs, was also highly similar compared to the pattern of dry mass (Table 5.4, Figure 5.4), except there was an interaction effect between wrack burial and macroinvertebrate presence for both the P content of the total plant and the total shoot: if wrack was buried, P content of the total plant

and the total shoot was higher in the presence of macroinvertebrates, while the opposite was the case when wrack was placed on the surface. In the presence of macroinvertebrates, P content of *C. maritima* was 3.1 ± 1.4 mg P against 0.9 ± 0.5 mg P for the total plant, 3.1 ± 1.4 P mg against 0.9 ± 0.5 mg P for the total shoot and 0.3 ± 0.1 mg P against 0.1 ± 0.1 mg P for the roots, when wrack was buried or placed on the surface, respectively. In the absence of

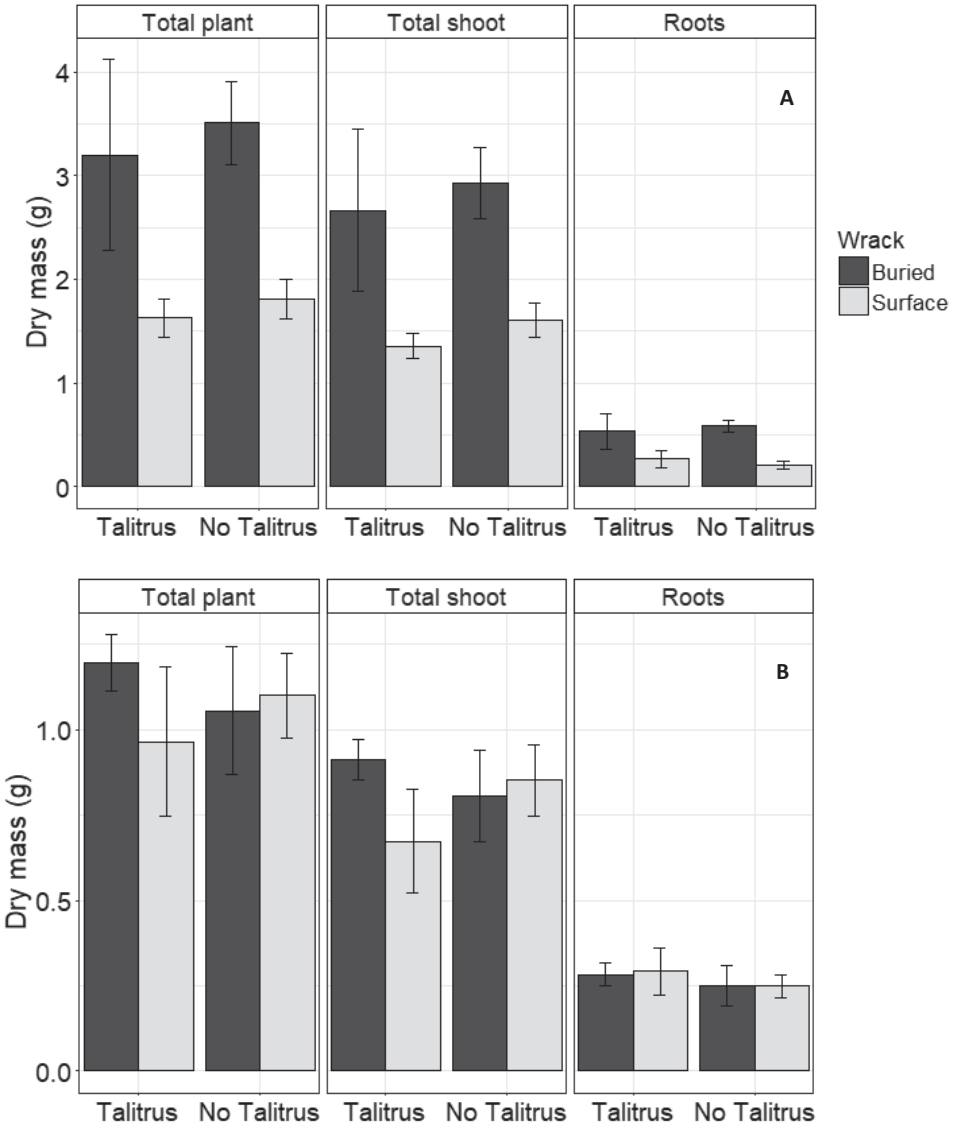

Figure 5.2. Dry mass of the total plant (roots and shoot), total shoot (stem and leaves) and roots of A) *Cakile maritima* and B) *Elytrigia juncea* at harvest shown for both buried or surface-exposed wrack and in the presence or absence of the amphipod *Talitrus saltator*. For buried wrack n=3, while n=6 for surface-exposed wrack in the presence of *T. saltator*. For all other treatment combinations n=8. Error bars indicate the standard error from the mean.

macroinvertebrates, P content of *C. maritima* was 2.1 ± 0.7 mg P against 1.1 ± 0.4 mg P for the total plant, 1.6 ± 0.5 P mg against 1.0 ± 0.4 mg P for the total shoot and 0.4 ± 0.2 mg P against 0.1 ± 0.1 mg P for the roots, when wrack was buried deep or placed on the surface, respectively. Thus, the P content was approximately two to three times as high when wrack was buried as opposed to surface wrack.

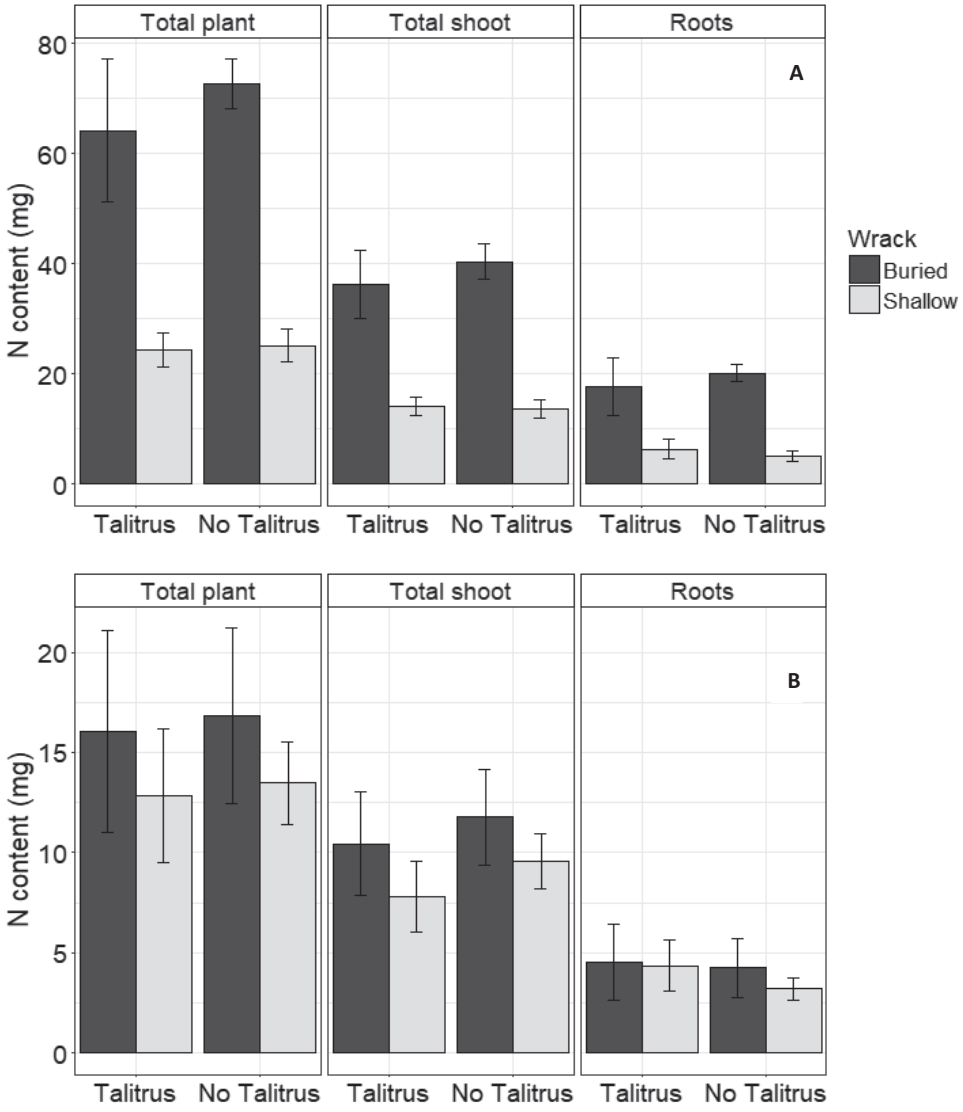

Figure 5.3. N content of the total plant (roots and shoot), total shoot (stem and leaves) and roots of A) *Cakile maritima* and B) *Elytrigia juncea* at harvest shown for both buried or surface-exposed wrack and in the presence or absence of the amphipod *Talitrus saltator*. For buried wrack n=3, while n=6 for surface-exposed wrack in the presence of *T. saltator*. For all other treatment combinations n=8. Error bars indicate the standard error from the mean.

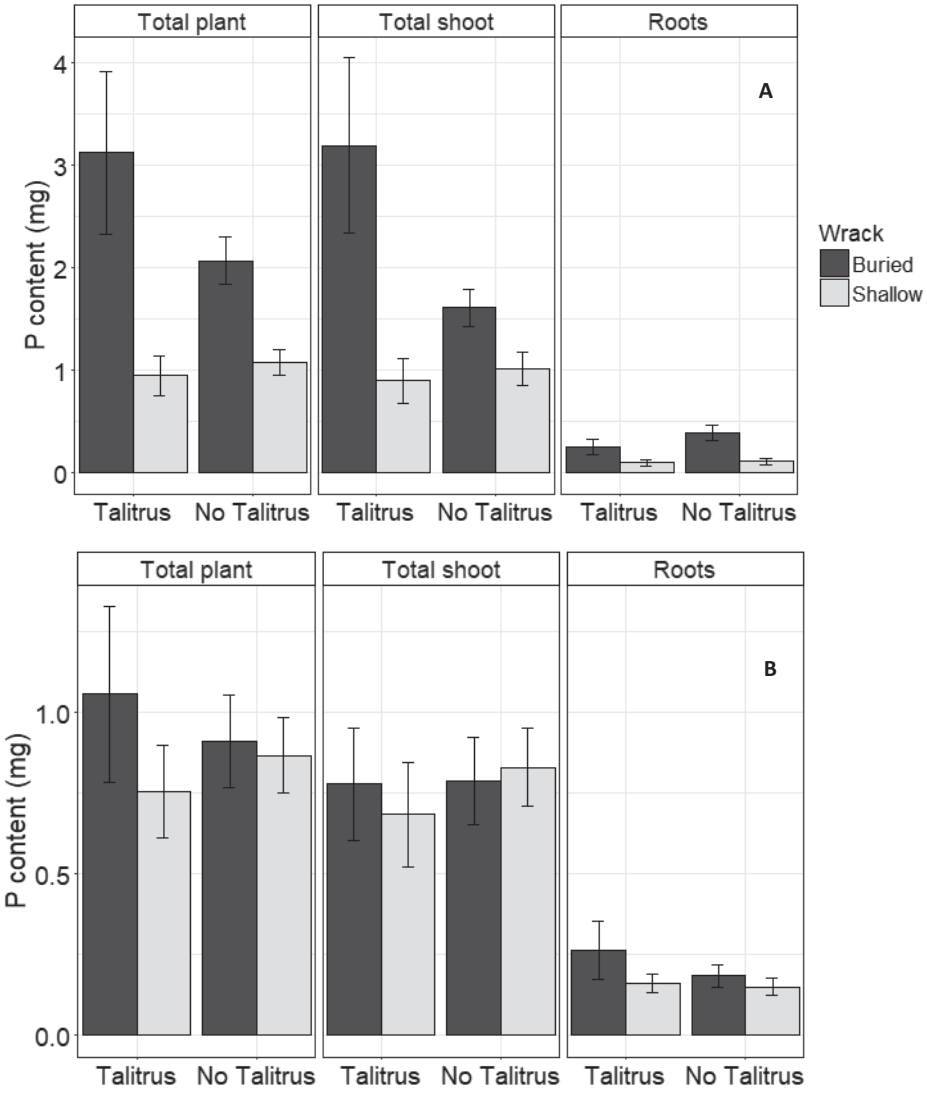

Figure 5.4. P content of the total plant (roots and shoot), total shoot (stem and leaves) and roots of A) *Cakile maritima* and B) *Elytrigia juncea* at harvest shown for both buried or surface-exposed wrack and in the presence or absence of the amphipod *Talitrus saltator*. For buried wrack n=3, while n=6 for surface-exposed wrack in the presence of *T. saltator*. For all other treatment combinations n=8. Error bars indicate the standard error from the mean.

Table 5.2. Overview of the two-way ANOVA results on the effect of wrack burial (Wrack) and macroinvertebrate presence or absence (Macroinvertebrates) on plant dry mass, for two species (*Cakile maritima* and *Elytrigia juncea*) and plant organs separately. An asterix indicates a significant p-value (<0.05).

		df	*F*	*p*
C. maritima				
Total plant	Wrack	1	20.2	<0.001 *
	Macroinvertebrates	1	1.8	0.19
	Wrack * Macroinvertebrates	1	0.0	0.87
Total shoot	Wrack	1	17.8	<0.001 *
	Macroinvertebrates	1	2.2	0.15
	Wrack * Macroinvertebrates	1	0.0	0.98
Roots	Wrack	1	21.7	<0.001 *
	Macroinvertebrates	1	0.3	0.60
	Wrack * Macroinvertebrates	1	0.5	0.47
E. juncea				
Total plant	Wrack	1	0.1	0.74
	Macroinvertebrates	1	0.1	0.74
	Wrack * Macroinvertebrates	1	1.3	0.26
Total shoot	Wrack	1	0.3	0.61
	Macroinvertebrates	1	0.5	0.48
	Wrack * Macroinvertebrates	1	2.0	0.18
Roots	Wrack	1	0.0	0.98
	Macroinvertebrates	1	0.5	0.50
	Wrack * Macroinvertebrates	1	0.0	0.93

Table 5.3. Overview of the two-way ANOVA results on the effect of wrack burial (Wrack) and macroinvertebrate presence or absence (Macroinvertebrates) on N content, for two species (*Cakile maritima* and *Elytrigia juncea*) and plant organs separately. An asterix indicates a significant p-value (<0.05), while an open circle indicates a trend (*p*<0.10).

		df	*F*	*p*
C. maritima				
Total plant	Wrack	1	88.2	<0.001 *
	Macroinvertebrates	1	5.3	0.03 *
	Wrack * Macroinvertebrates	1	0.6	0.46
Total shoot	Wrack	1	78.1	<0.001 *
	Macroinvertebrates	1	3.7	0.07 °
	Wrack * Macroinvertebrates	1	0.6	0.45
Roots	Wrack	1	52.6	<0.001 *
	Macroinvertebrates	1	1.6	0.21
	Wrack * Macroinvertebrates	1	0.8	0.37
E. juncea				
Total plant	Wrack	1	0.7	0.43
	Macroinvertebrates	1	0.2	0.69
	Wrack * Macroinvertebrates	1	0.1	0.76
Total shoot	Wrack	1	1.4	0.26
	Macroinvertebrates	1	1.1	0.30
	Wrack * Macroinvertebrates	1	0.2	0.67
Roots	Wrack	1	0.1	0.71
	Macroinvertebrates	1	0.4	0.56
	Wrack * Macroinvertebrates	1	0.0	0.94

Table 5.4. Overview of the two-way ANOVA results on the effect of wrack burial (Wrack) and macroinvertebrate presence or absence (Macroinvertebrates) on P content, for two species (*Cakile maritima* and *Elytrigia juncea*) and plant organs separately. An asterix indicates a significant p-value (<0.05).

		df	*F*	*p*
C. maritima				
Total plant	Wrack	1	27.9	<0.001 *
	Macroinvertebrates	1	0.1	0.71
	Wrack * Macroinvertebrates	1	4.5	<0.05 *
Total shoot	Wrack	1	18.5	<0.001 *
	Macroinvertebrates	1	1.6	0.22
	Wrack * Macroinvertebrates	1	8.8	<0.001 *
Roots	Wrack	1	20.4	<0.001 *
	Macroinvertebrates	1	2.5	0.13
	Wrack * Macroinvertebrates	1	0.2	0.63
E. juncea				
Total plant	Wrack	1	0.7	0.41
	Macroinvertebrates	1	0.0	0.84
	Wrack * Macroinvertebrates	1	0.6	0.43
Total shoot	Wrack	1	0.0	0.96
	Macroinvertebrates	1	0.5	0.50
	Wrack * Macroinvertebrates	1	0.5	0.48
Roots	Wrack	1	2.3	0.14
	Macroinvertebrates	1	0.6	0.46
	Wrack * Macroinvertebrates	1	0.7	0.40

5.5 Discussion

In this study, we focused on the effect of wrack burial and macroinvertebrate presence on decomposition and nutrient availability for beach pioneer plant growth. We found a differential effect of wrack burial on plant growth. While burial of wrack had a strong positive effect on the growth of *Cakile maritima*, no significant effect was found for *Elytrigia juncea*. For *C. maritima*, the N content of the total plant was lower in the presence of macroinvertebrates. For buried wrack, P content was higher for both the total plant and total shoot in the presence of macroinvertebrates. Differences in N and P content of plants due to macroinvertebrate presence did however not result in differences in plant dry mass. Together, this suggests that macroinvertebrates enhance decomposition of wrack, but that released inorganic N is taken up by the microbial community or is forming complexes with phenolic compounds, resulting in immobilisation of N for plants. Excess P on the other hand was incorporated in *C. maritima*. We conclude that the burial of wrack is of paramount importance for *C. maritima* growth in support of embryo dune formation and possibly further ecological development of the sandy beach and dune ecosystems.

5.5.1 Buried wrack is a strong driver of Cakile maritima *growth*

As to our first hypothesis, buried wrack had indeed a strong positive effect on beach pioneer plant growth, but only for *C. maritima*. *Elytrigia juncea* plants, on the other hand, did not respond to wrack presence, irrespective of burial depth. For *C. maritima*, dry mass and P content were two times, and N content three times as high when wrack was buried as opposed to surface wrack. Thus, *C. maritima* plants not only accumulated more nutrients both in the total shoot and roots, but also grew larger when wrack was buried. It is expected that the differences in plant growth between *C. maritima* and *E. juncea* are mainly species-specific and related to differences in growth and habitat requirements. *Cakile maritima* is an annual herb, and annual plant species are known to exhibit higher and faster plant growth of both the shoot and roots than perennial species (Gross et al. 1992). In contrast, *Elytrigia juncea* is a perennial grass (Hanlon and Mesgaran 2014) and may thus exhibit lower plant growth as opposed to *C. maritima*. As *C. maritima* is typically associated with drift lines (Davy et al. 2006), while *E. juncea* can be found at the dune foot or in stabilised stands with *C. maritima* (Speybroeck et al. 2008a, Davy et al. 2006), this may also be related to their, only partly overlapping, habitat needs. Buried wrack may thus promote the growth of some plant species more profoundly than other species on the beach, which ultimately may result in an altered plant community structure on sandy beaches.

Our findings suggest that the positive effect of buried wrack on *C. maritima* growth is primarily due to an increase in sediment moisture content rather than an increase in nutrient availability. To discriminate between the moisture and nutrient effect of wrack on plant growth, we included nutrient addition treatments. These treatments showed that in general beach pioneer plants accumulated more N and P when a nutrient solution was added than when plants were grown without nutrient additions, indicating that *C. maritima* benefited from additional N and P in terms of nutrient accumulation (see Appendix, Figure A5.3-S5.5 and Table A5.2). The additional nutrients did, however, not directly translate into an increase in plant growth (Table A5.2, Figure A5.1). This indicates that nutrients are not a limiting factor for plant growth. Indeed, plant species that are adapted to low nutrient conditions have a low growth rate, even if nutrient availability is increased (Chapin III et al. 1990). Only when buried wrack was added as opposed to solely nutrients in solution, the increased accumulation of N and P did result in higher plant growth, suggesting that buried wrack had an additive positive effect. In our study, buried wrack had a higher moisture content than surface-facing wrack. Buried wrack remains moist as it is protected from severe desiccation by a layer of sand. Higher soil moisture contents are therefore an important wrack-mediated factor, positively influencing *C. maritima* plant growth. The decay of wrack is greater when the material is fresh and moist (Coûteaux et al. 1995), thereby releasing more dissolved organic carbon and increasing microbial activity (Coupland et al. 2007, Lavery et al. 2013). The higher the microbial activity, the faster remineralisation by bacteria occurs and nutrients become available for plant uptake (Colombini and Chelazzi 2003). In this study, this seems particularly true for the release of P. Moreover, by retaining moisture, wrack forms an organic band of easy-to-reach moisture in the sand column (Adair et al. 1990), which benefits beach pioneer plant growth (Del Vecchio et al. 2013), most likely by lifting moisture limitation and promoting N and P transport and uptake (Aerts and Chapin III 1999). This is opposed to rain water entering the

sand and quickly moving downwards beyond the reach of the roots, as sand has a low moisture holding capacity (Pakeman and Lee 1991a). We conclude that burial of wrack has a positive effect on *C. maritima* plant growth mainly through enhancement of sediment moisture content.

5.5.2 Macroinvertebrate presence affects plant nutrient content, but not plant growth

Macroinvertebrate presence had an effect on N and P content in plant tissue but not on beach pioneer plant growth, which is in contrast to our second hypothesis. In addition, we found some interactive effects between wrack burial and macroinvertebrate presence for N and P content in plant tissue only, which is partly in line with our final hypothesis. Thus, macroinvertebrates were important in decomposition-driven nutrient availability. Previous studies on the effect of supratidal macroinvertebrates on wrack decomposition reported mixed results, ranging from large positive (Lastra et al. 2008, Salathé and Riera 2012) to small or no effects (Inglis 1989, Jędrzejczak 2002b, Catenazzi and Donnelly 2007) and our findings are in agreement to the former studies.

For *C. maritima*, a negative effect of macroinvertebrate presence on the N content of the total plant was observed, which appears to be in contrast to our second hypothesis. In other nutrient-poor ecosystems, such as northern peatlands, high decomposing activities however result in an initial immobilisation of N by the microbial community (Dorrepaal et al. 2007). Macroinvertebrates enhance decomposition via fragmentation of wrack (Ince et al. 2007, Salathé and Riera 2012, Lastra et al. 2015), but the additionally released N may initially be incorporated in microbial instead of plant biomass (Dorrepaal et al. 2007). In addition, phenolic compounds, which are present in high amounts in *Fucus* sp. (Targett et al. 1992), may be released during decomposition. This released carbon may have acted as a carbon source for the microbial community, enhancing its activity (Lavery et al. 2013) and consequently N immobilisation (Michelsen et al. 1995). Released phenolic compounds may however also bind to inorganic N thereby forming insoluble complexes and causing chemical immobilisation of N (Hättenschwiler and Vitousek 2000). On the other hand, P content was higher for both the total plant and total shoot in the presence of macroinvertebrates when wrack was buried, while the opposite was the case in the presence of macroinvertebrates when wrack was placed on the surface. Macroinvertebrates thus had a positive effect on decomposition-driven nutrient availability, supporting a higher P content in *C. maritima* plants, but only when moisture was not limiting. Differences in N and P content of *C. maritima* were only observed when wrack was buried and sand moisture content was increased, which is likely due to an increase in microbial activity (Coupland et al. 2007, Lavery et al. 2013) as opposed to drier surface-facing wrack. In addition, moist wrack was probably more palatable to *T. saltator* resulting in a higher consumption (see Ruiz-Delgado et al. 2015). The findings for P further suggest that the microbial community associated with wrack may be principally N (or C) limited and less limited by P (e.g. Heuck et al. 2015), resulting in an increased uptake of N when this became available during wrack decomposition. Plants appeared to be P limited in the absence of macroinvertebrates when wrack was buried, as the N/P ratio of *C. maritima* shoots was relatively high (27.7 ± 12.1; Figure A5.1, Table A5.1) and well above a N/P ratio of 16 which indicates a P limitation (Aerts and Chapin III 1999). Plants may in that case have

competed more strongly with the microbial community for P than N, resulting in a higher P content and lower N/P ratio (12.5 ± 4.3; Figure A5.1, Table A5.1) when macroinvertebrates were present and nutrient availability was enhanced. In contrast, *C. maritima* was limited by N and not P in other experiments (Pakeman and Lee 1991a, Pakeman and Lee 1991b), although the interactive effects between wrack, moisture, nutrients, the microbial community and the macroinvertebrate community were not taken into account in those studies. The final uptake of N and P by *C. maritima* plants thus may potentially be influenced by the competitive ability of both the microbial community and the individual plant for these nutrients. Neither for N or P though, an effect of macroinvertebrate presence was found on plant dry mass. This suggests that lifting P limitation is not sufficient to result in an increase in plant growth, even though total moisture content in the sediment increased when wrack was buried. Moisture was either still limiting or an additional factor had been limiting plant growth (e.g. salt, micronutrients). Therefore, our study highlights the complexity of macroinvertebrate-mediated processes that result in the degradation of wrack and subsequent uptake of nutrients by beach pioneer plants.

It should be noted that the effects of biotic factors were likely underestimated in our experiment due to the considerable mortality of *T. saltator*. The effects of macroinvertebrate presence were even smaller when mesocosms in which no macroinvertebrates were retrieved upon harvest were included in the analysis (see Appendix, Figure A5.6-A5.9 and Table A5.3-A5.6). Although we had used a representative *T. saltator* density in this experiment, the effect of *T. saltator* on decomposition-driven nutrient availability may be larger in the field where *T. saltator* densities can locally become very high (up to 3500 individuals m^{-2} (Van Colen et al. 2006) or even 7900 individuals m^{-2} (Ruiz-Delgado et al. 2016)). The effect of macroinvertebrate presence on decomposition-driven nutrient availability may thus be amplified when more individuals are present and active. Nevertheless, this will likely not result in an increase in plant growth as other factors appear to limit plant growth.

Together this suggests that wrack burial and consumption by macroinvertebrates are both drivers of decomposition-driven nutrient availability and subsequently change the nutrient dynamics of beach pioneer plants. As only wrack burial resulted in a higher plant growth, it appears that beach pioneer plants are limited by moisture rather than nutrients. Macroinvertebrates may have a positive effect on plant growth when decomposition and nutrient release, due to macroinvertebrate feeding activity, exceed the nutritional needs of the microbial community, or in the long term when nutrients are finally released from the microbial community via remineralisation.

5.5.3 Conclusions

We conclude that wrack burial enhances nutrient availability and stimulates the growth of some beach pioneer plants, especially *C. maritima*. Beach pioneer plants appeared to be mainly limited by moisture rather than nutrients. Decomposition of wrack by macroinvertebrates appears to be an additional factor that increased nutrient availability, but this does not result in an increase in plant growth. Leaving wrack on the beach and allowing it to be covered by sand and subsequently decomposed, provides a moisture and nutrient hot spot for beach pioneer plants on sandy beaches. Buried wrack can provide preferable

microclimate conditions for plant growth and possibly support embryo dune formation, e.g. for *C. maritima* (Davy et al. 2006), while also having a positive effect on dune vegetation in terms of plant diversity (Del Vecchio et al. 2017). It is therefore recommended to allow wrack to be deposited and buried on the sandy beach to promote the sandy beach ecosystem in future coastal management strategies.

5.6 Appendix

5.6.1 N/P ratio for the main treatments

For *C. maritima*, there was a significant effect of wrack burial and a significant interaction effect between wrack burial and macroinvertebrate presence for both N/P ratio of the total plant and the total shoot (Table A5.1, Figure A5.1). For buried wrack, the N/P ratio of the total plant was higher in the absence (N/P = 37.5 ± 11.7) than in the presence (N/P = 21.5 ± 4.4) of macroinvertebrates. The opposite was the case for surface-facing wrack, where total plant N/P ratio was higher in the presence (N/P = 27.8 ± 7.7) than in the absence (N/P = 23.4 ± 5.0) of macroinvertebrates. Similar to the total plant, the N/P ratio of the total shoot was higher in the absence (N/P = 27.7 ± 12.1) than in the presence (N/P = 12.5 ± 4.3) of macroinvertebrates when wrack was buried. The opposite was observed with surface-facing wrack, where the N/P ratio of the total shoot was higher in the presence (N/P = 17.6 ± 4.9) than in the absence (N/P = 14.2 ± 3.7) of macroinvertebrates. There were no significant interaction effects on the N/P ratio of the roots, but the N/P ratio of the roots (N/P = 64.8 ± 34.0) was three times higher than in the total shoot (N/P = 18.0 ± 10.2). For *E. juncea*, there were no significant differences of wrack position or macroinvertebrate presence on the N/P ratio of either the total plant, total shoot or roots (Table A5.1, Figure A5.1).

Table A5.1. Overview of the two-way ANOVA results on the effect of wrack burial (Wrack) and macroinvertebrate presence or absence (Macroinvertebrates) on N/P ratio, for two species (*Cakile maritima* and *Elytrigia juncea*) and plant organs separately. An asterix indicates a significant p-value (<0.05).

		df	*F*	*p*
C. maritima				
Total plant	Wrack	1	4.6	<0.05 *
	Macroinvertebrates	1	1.9	0.18
	Wrack * Macroinvertebrates	1	9.8	<0.01 *
Total shoot	Wrack	1	4.7	0.04 *
	Macroinvertebrates	1	2.5	0.13
	Wrack * Macroinvertebrates	1	11.2	<0.01 *
Roots	Wrack	1	0.5	0.48
	Macroinvertebrates	1	0.8	0.37
	Wrack * Macroinvertebrates	1	0.0	0.89
E. juncea				
Total plant	Wrack	1	0.1	0.73
	Macroinvertebrates	1	0.1	0.79
	Wrack * Macroinvertebrates	1	0.3	0.61
Total shoot	Wrack	1	2.4	0.14
	Macroinvertebrates	1	0.6	0.45
	Wrack * Macroinvertebrates	1	0.5	0.51
Roots	Wrack	1	0.5	0.50
	Macroinvertebrates	1	0.0	0.97
	Wrack * Macroinvertebrates	1	0.6	0.45

5.6.2 Results nutrient addition test

For shoot, root and total plant dry mass, no significant differences among the nutrient treatments were found for either *Cakile maritima* or *Elytrigia juncea* (Table A5.2, Figure A5.2).

The N content was significantly higher in *C. maritima* that received a nutrient solution (total plant: 17.7 ± 4.3 mg N, total shoot: 14.8 ± 3.5 mg N) than plants grown with inoculum (total plant: 9.4 ± 3.8 mg N, p=0.006; total shoot: 5.2 ± 2.0 mg N; p<0.001) or without nutrient

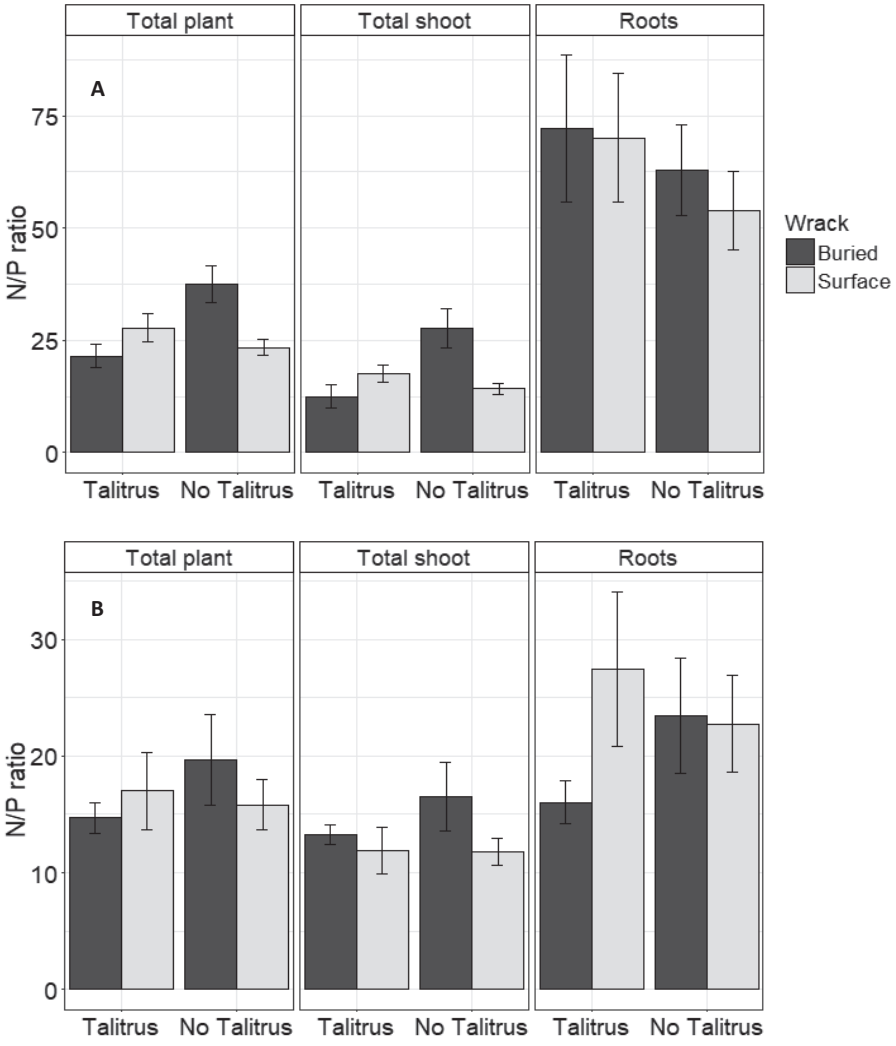

Figure A5.1. N/P ratio of the total plant (roots and shoot), total shoot (stem and leaves) and roots of A) *Cakile maritima* and B) *Elytrigia juncea* shown for both buried or surface-exposed wrack and in the presence or absence of the amphipod *Talitrus saltator*. For buried wrack n=3, while n=6 for surface-exposed wrack in the presence of *T. saltator*. For all other treatment combinations n=8. Error bars indicate the standard error from the mean.

additions (total plant: 7.5 ± 1.2 mg N, p=0.001; total shoot: 4.3 ± 0.6 mg N, p<0.001; Table A5.2, Figure A5.3). For *E. juncea*, N content was significantly higher in plants that received a nutrient solution (total plant: 14.7 ± 6.5 mg N, total shoot: 10.9 ± 5.1 mg N) compared to plants grown with inoculum (total plant: 6.9 ± 2.1 mg N, p=0.03; total shoot: 5.2 ± 1.6 mg N, p=0.04), while plants that received a nutrient solution only showed a trend towards a higher N content than plants grown without nutrient additions (total plant: 8.6 ± 2.7 mg N, p=0.09; total shoot:

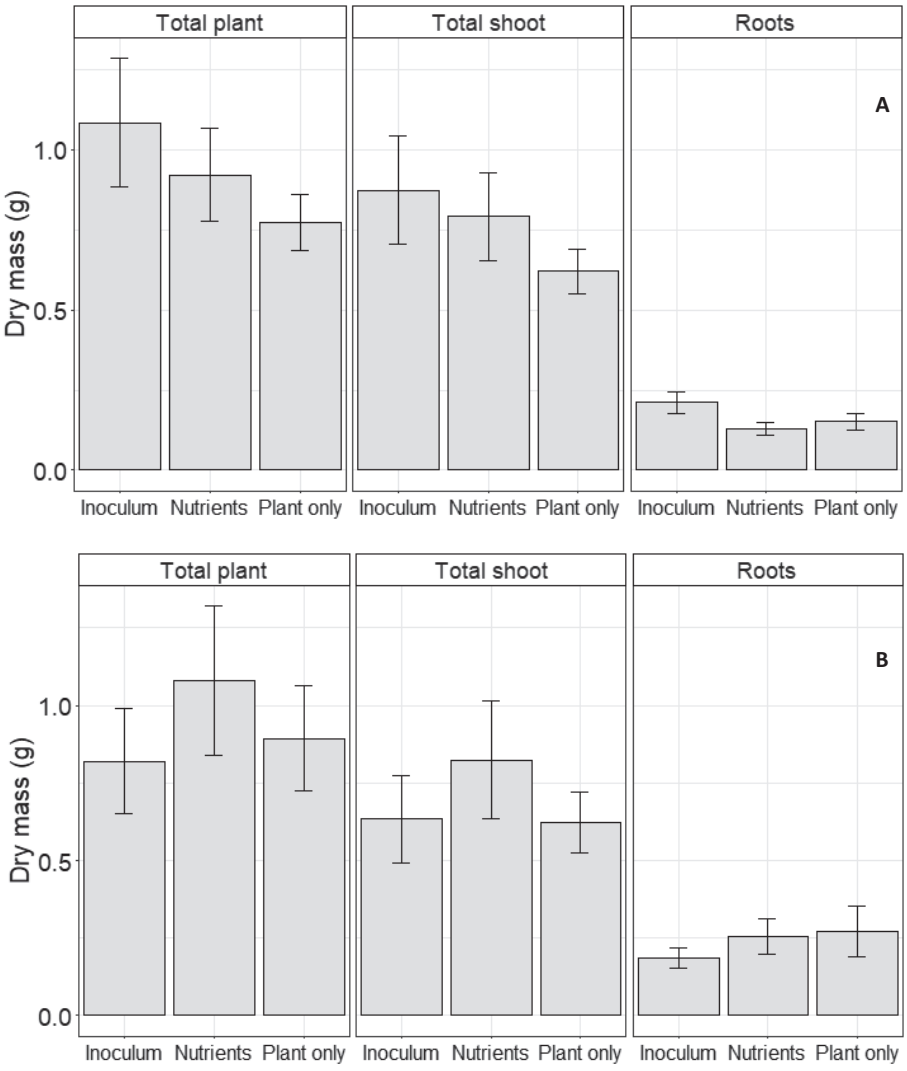

Figure A5.2. Absolute dry mass of the total plant (roots and shoot), total shoot (stem and leaves) and roots of A) *Cakile maritima* and B) *Elytrigia juncea* at harvest shown for the three treatments in the nutrient addition test: plant only, added nutrient solution equivalent to 100% wrack in trial (Nutrients) and added inoculum based on fresh wrack (Inoculum). For all treatments n=5. Error bars indicate the standard error from the mean.

5.9 ± 1.2 mg N, p=0.06; Table A5.2, Figure A5.3). Thus, for both plant species, the N content was approximately two to three times higher when plants received a nutrient solution as opposed to plants receiving either inoculum or no nutrient additions.

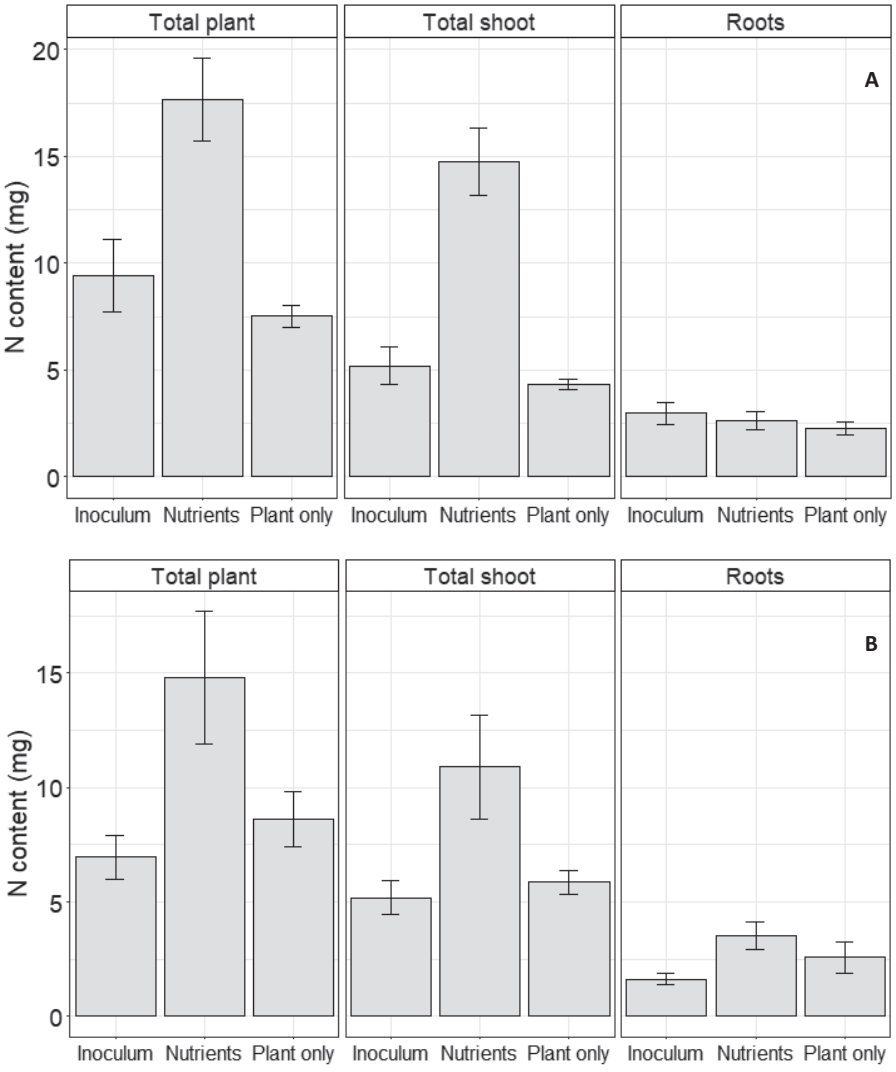

Figure A5.3. N content of the total plant (roots and shoot), total shoot (stem and leaves) and roots of A) *Cakile maritima* and B) *Elytrigia juncea* at harvest shown for the three treatments in the nutrient addition test: plant only, added nutrient solution equivalent to 100% wrack in trial (Nutrients) and added inoculum based on fresh wrack (Inoculum). For all treatments n=5. Error bars indicate the standard error from the mean.

The P content was the only measured variable that was significantly different in the roots (Table A5.2, Figure A5.4). For *C. maritima*, the P content was significantly higher in plants that received a nutrient solution (total plant: 1.7 ± 0.4 mg P, total shoot: 1.6 ± 0.2 mg P, roots: 0.2 ± 0.1 mg P) compared to plants grown with inoculum (total plant: 0.9 ± 0.3 mg P, p=0.004; total shoot: 0.9 ± 0.3 mg P, p=0.005; roots: 0.1 ± 0.0 mg P, p=0.02) or without nutrient additions (total plant: 0.8 ± 0.3 mg P, p=0.002; total shoot: 0.8 ± 0.3 mg P, p=0.001; roots: 0.1

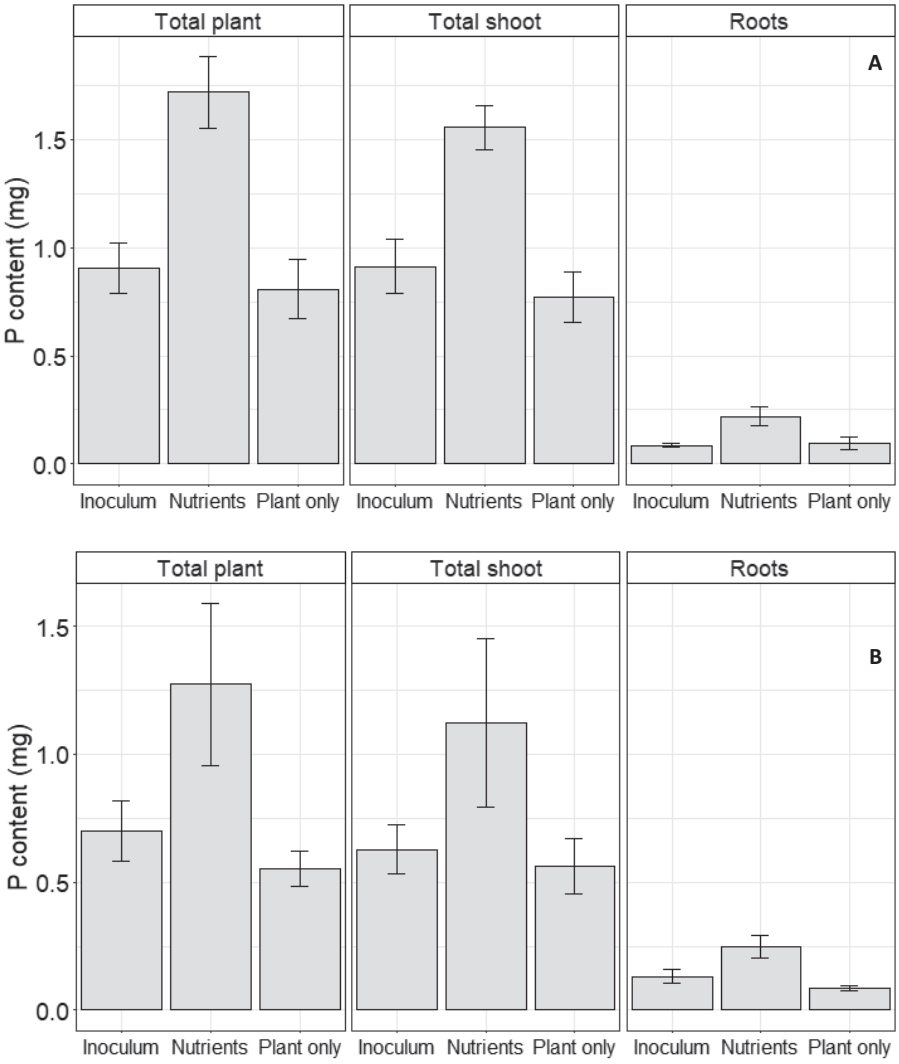

Figure A5.4. P content of the total plant (roots and shoot), total shoot (stem and leaves) and roots of A) *Cakile maritima* and B) *Elytrigia juncea* shown for the three treatments in the nutrient addition test: plant only, added nutrient solution equivalent to 100% wrack in trial (Nutrients) and added inoculum based on fresh wrack (Inoculum). For all treatments n=5. Error bars indicate the standard error from the mean.

± 0.1 mg P, p=0.03). For *E. juncea*, the P content was only significantly higher in the roots of plants that received a nutrient solution (0.2 ± 0.1 mg P) compared to plants grown with inoculum (0.1 ± 0.1 mg P, p<0.05) or without nutrient additions (0.1 ± 0.0 mg P, p=0.007). Thus, for both species, the P content was approximately two times as high when plants received a nutrient solution as opposed to plants receiving either inoculum or no nutrient additions.

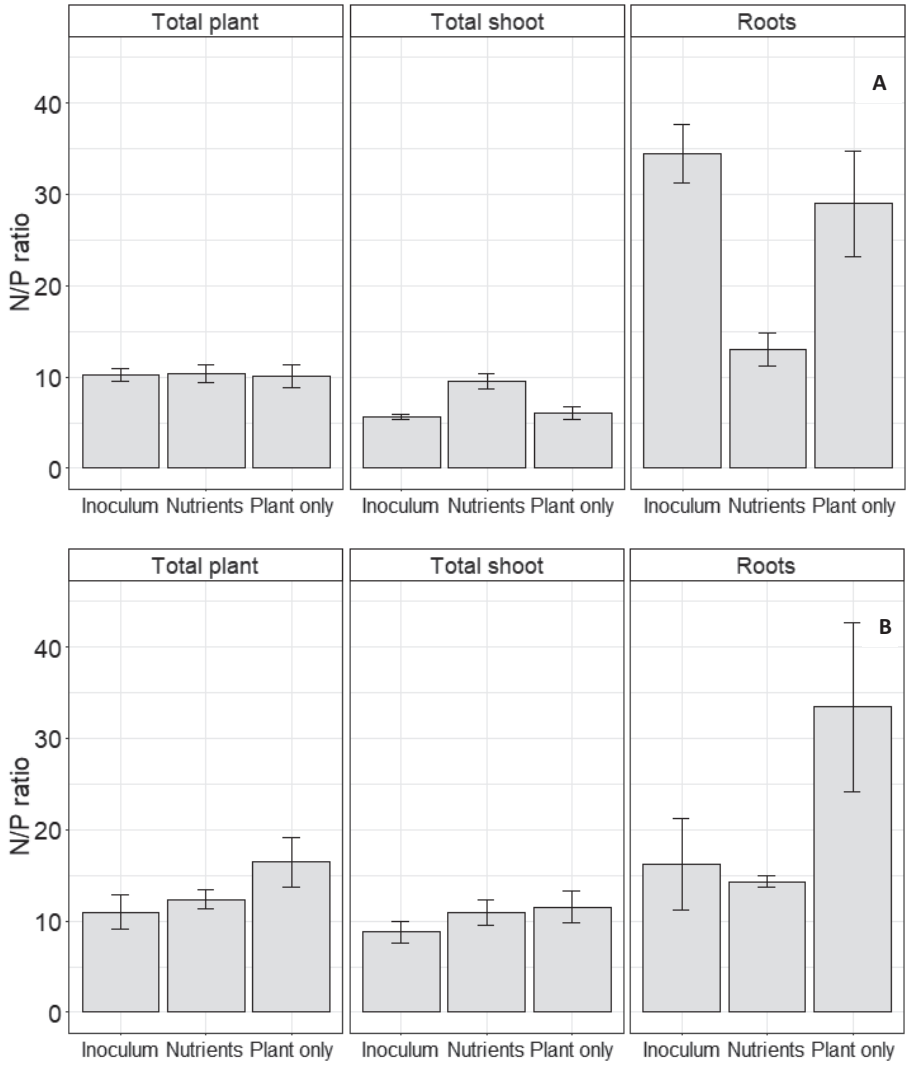

Figure A5.5. N/P ratio of the total plant (roots and shoot), total shoot (stem and leaves) and roots of A) *Cakile maritima* and B) *Elytrigia juncea* shown for the three treatments in the nutrient addition test: plant only, added nutrient solution equivalent to 100% wrack in trial (Nutrients) and added inoculum based on fresh wrack (Inoculum). For all treatments n=5. Error bars indicate the standard error from the mean.

Table A5.2. Overview of the one-way ANOVA results on the treatment effect in the nutrient addition test (nutrient solution, inoculum and plant only) on dry mass, N and P content and N/P ratio, for each plant species and plant organ (total plant, total shoot and roots) separately. An asterix indicates a significant p-value (<0.05), while an open circle indicates a trend (p<0.10).

		df	*F*	*p*
Dry mass	*C. maritima*			
	Total plant	2	1.1	0.38
	Total shoot	2	1.0	0.41
	Roots	2	2.4	0.14
	E. juncea			
	Total plant	2	0.5	0.64
	Total shoot	2	0.6	0.57
	Roots	2	0.6	0.58
N content	*C. maritima*			
	Total plant	2	12.7	0.001 *
	Total shoot	2	29.8	<0.001 *
	Roots	2	0.7	0.50
	E. juncea			
	Total plant	2	4.7	0.03 *
	Total shoot	2	5.4	0.02 *
	Roots	2	3.1	0.08 °
P content	*C. maritima*			
	Total plant	2	12.8	0.001 *
	Total shoot	2	13.4	<0.001 *
	Roots	2	5.9	0.02 *
	E. juncea			
	Total plant	2	3.7	0.06 °
	Total shoot	2	2.2	0.16
	Roots	2	7.5	0.008 *
N/P ratio	*C. maritima*			
	Total plant	2	0.03	0.97
	Total shoot	2	10.9	0.002 *
	Roots	2	7.84	0.007 *
	E. juncea			
	Total plant	2	2.06	0.17
	Total shoot	2	0.96	0.41
	Roots	2	3.00	0.09 °

The N/P ratio of the total shoot was significantly higher in *C. maritima* that received a nutrient solution (N/P = 9.5 ± 1.8) than those grown with inoculum (N/P = 5.6 ± 0.7, p=0.003) or without nutrient additions (N/P = 6.0 ± 1.6, p=0.007; Table A5.2, Figure A5.4). In contrast, the N/P ratio of the roots was significantly lower in *C. maritima* that received a nutrient solution (N/P = 13.0 ± 4.0) than those grown with inoculum (N/P = 34.4 ± 7.2; p=0.007) or without nutrient additions (N/P = 29.0 ± 13.0, p=0.037). For *E. juncea*, there were no significant differences between the treatments in the nutrient addition test for the N/P ratio of either the total plant, total shoot or root (Table A5.2, Figure A5.5).

5.6.3 Analyses performed on all mesocosms

In the following analyses, all mesocosms in the experiment were included, also those mesocosms to which macroinvertebrates were added, but none were retrieved upon harvest.

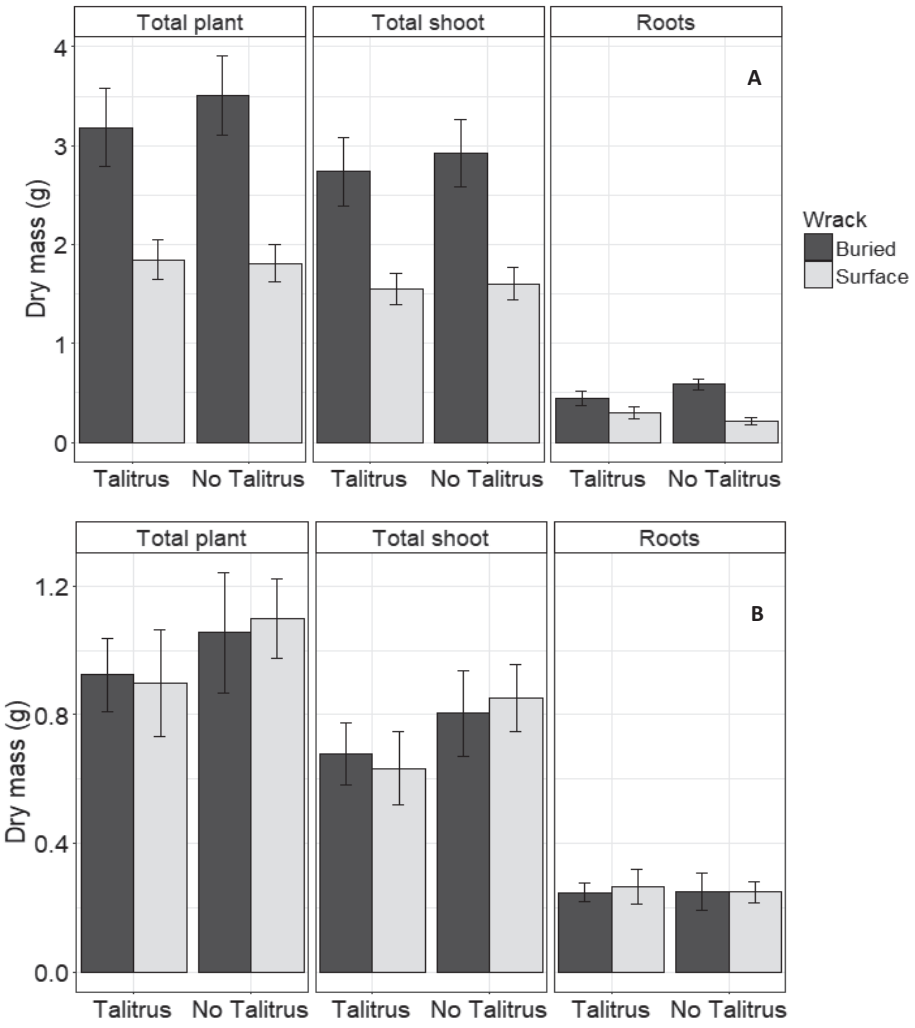

Figure A5.6. Dry mass of the total plant (roots and shoot), total shoot (stem and leaves) and roots of A) *Cakile maritima* and B) *Elytrigia juncea* at harvest shown for both buried or surface-exposed wrack and in the presence or absence of the amphipod *Talitrus saltator*. For all treatments n=8. Error bars indicate the standard error from the mean.

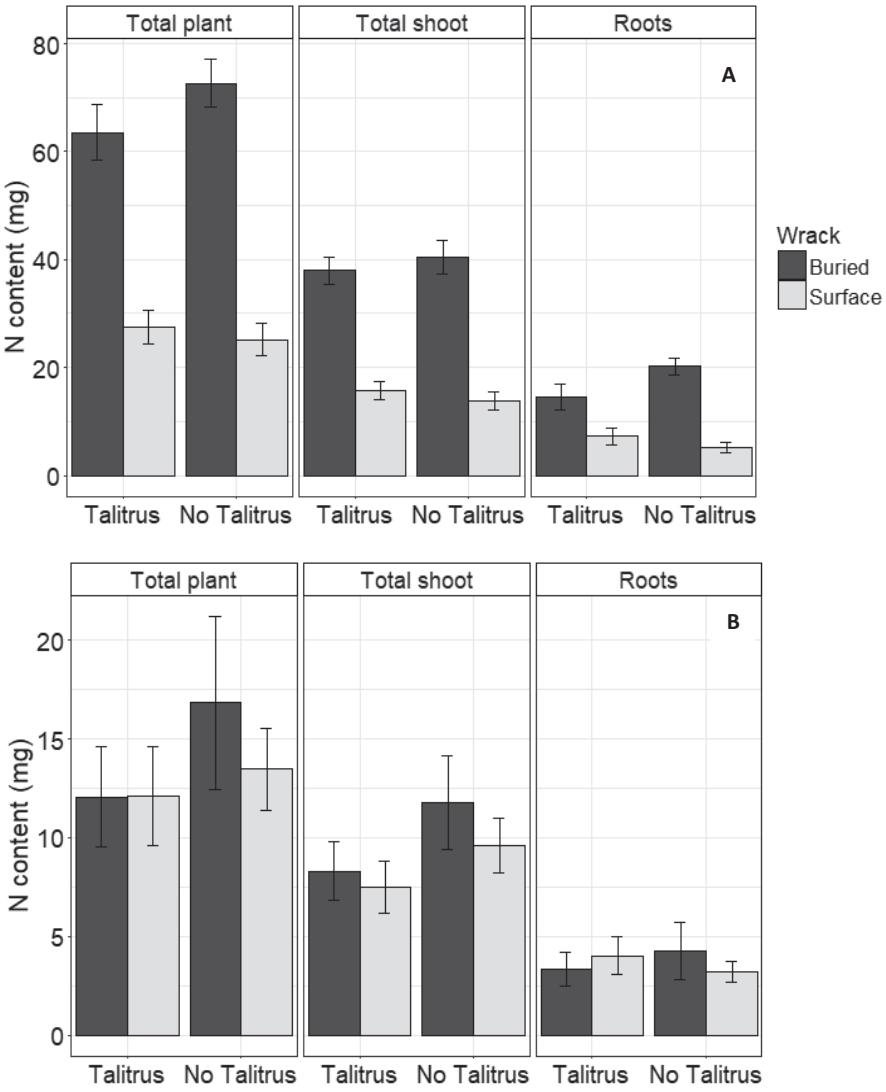

Figure A5.7. N content of the total plant (roots and shoot), total shoot (stem and leaves) and roots of A) *Cakile maritima* and B) *Elytrigia juncea* at harvest shown for both buried or surface-exposed wrack and in the presence or absence of the amphipod *Talitrus saltator*. For all treatments n=8. Error bars indicate the standard error from the mean.

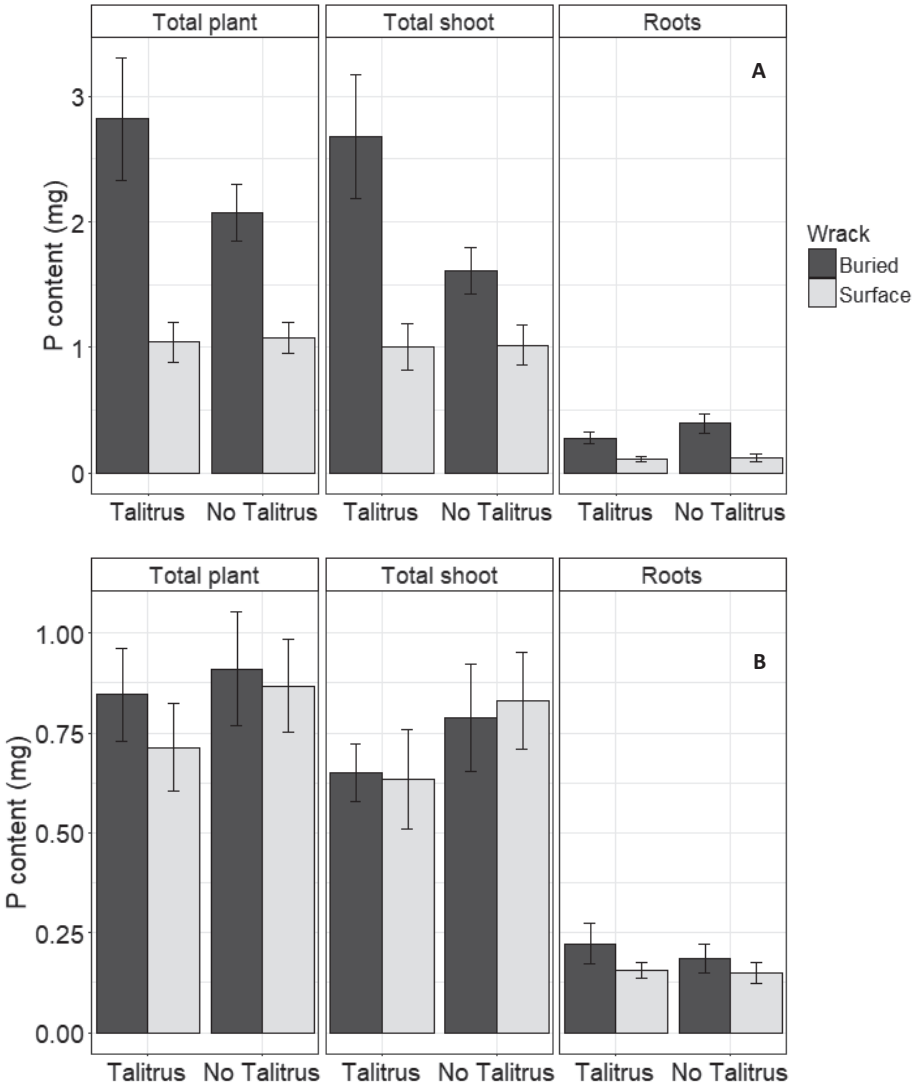

Figure A5.8. P content of the total plant (roots and shoot), total shoot (stem and leaves) and roots of A) *Cakile maritima* and B) *Elytrigia juncea* at harvest shown for both buried or surface-exposed wrack and in the presence or absence of the amphipod *Talitrus saltator*. For all treatments n=8. Error bars indicate the standard error from the mean.

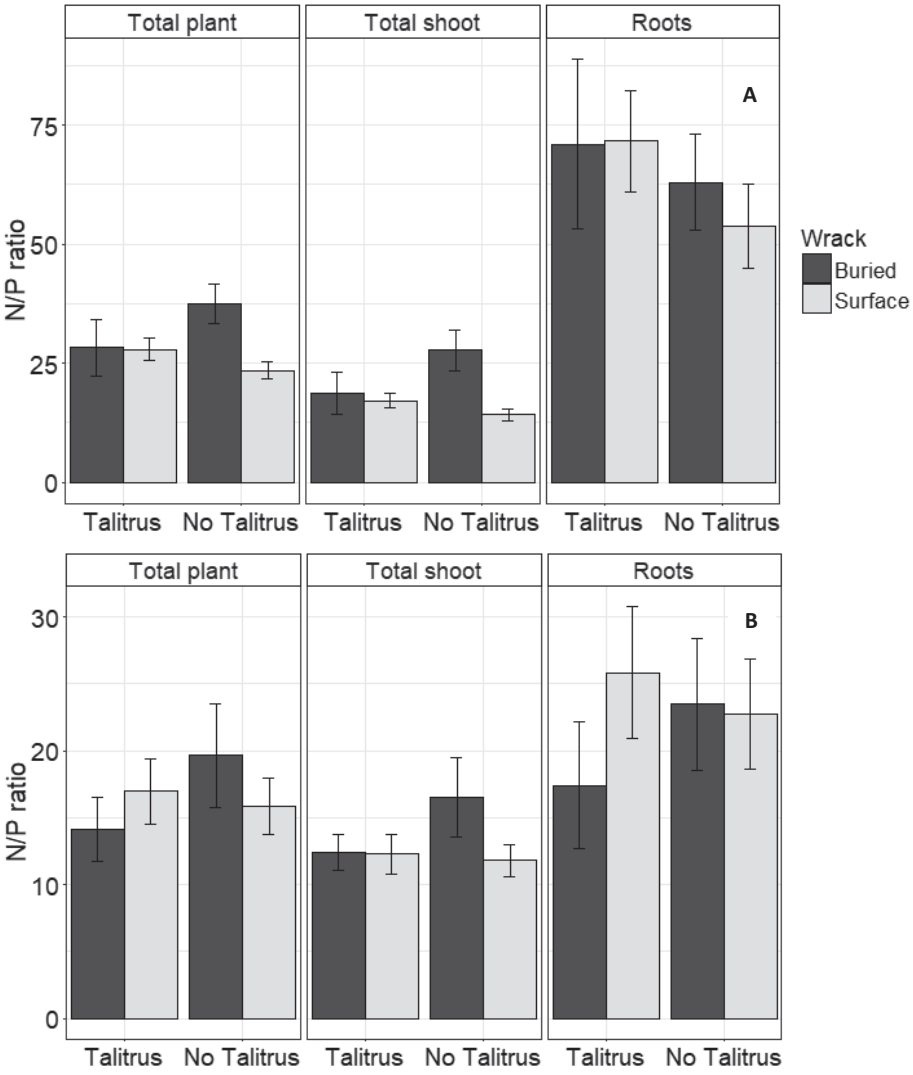

Figure A5.9. N/P ratio of the total plant (roots and shoot), total shoot (stem and leaves) and roots of A) *Cakile maritima* and B) *Elytrigia juncea* shown for both buried or surface-exposed wrack and in the presence or absence of the amphipod *Talitrus saltator*. For all treatments n=8. Error bars indicate the standard error from the mean.

Table A5.3. Overview of the two-way ANOVA results on the effect of wrack burial (Wrack) and macroinvertebrate presence or absence (Macroinvertebrates) on plant dry mass, for two species (*Cakile maritima* and *Elytrigia juncea*) and plant organs separately. An asterix indicates a significant p-value (<0.05), while an open circle indicates a trend (*p*<0.10).

		df	*F*	*p*
C. maritima				
Total plant	Wrack	1	23.6	<0.001 *
	Macroinvertebrates	1	0.2	0.64
	Wrack * Macroinvertebrates	1	0.3	0.57
Total shoot	Wrack	1	20.4	<0.001 *
	Macroinvertebrates	1	0.2	0.66
	Wrack * Macroinvertebrates	1	0.1	0.78
Roots	Wrack	1	19.1	<0.001 *
	Macroinvertebrates	1	0.2	0.69
	Wrack * Macroinvertebrates	1	3.7	0.07 °
E. juncea				
Total plant	Wrack	1	0.0	0.97
	Macroinvertebrates	1	1.6	0.22
	Wrack * Macroinvertebrates	1	0.3	0.61
Total shoot	Wrack	1	0.0	0.99
	Macroinvertebrates	1	2.9	0.10 °
	Wrack * Macroinvertebrates	1	0.3	0.61
Roots	Wrack	1	0.0	0.83
	Macroinvertebrates	1	0.0	0.83
	Wrack * Macroinvertebrates	1	0.0	0.86

Table A5.4. Overview of the two-way ANOVA results on the effect of wrack burial (Wrack) and macroinvertebrate presence or absence (Macroinvertebrates) on N content, for two species (*Cakile maritima* and *Elytrigia juncea*) and plant organs separately. An asterix indicates a significant p-value (<0.05), while an open circle indicates a trend (p<0.10).

		df	*F*	*p*
C. maritima				
Total plant	Wrack	1	105.3	<0.001 *
	Macroinvertebrates	1	0.7	0.41
	Wrack * Macroinvertebrates	1	1.9	0.17
Total shoot	Wrack	1	107.7	<0.001 *
	Macroinvertebrates	1	0.0	0.90
	Wrack * Macroinvertebrates	1	0.9	0.35
Roots	Wrack	1	44.9	<0.001 *
	Macroinvertebrates	1	1.1	0.30
	Wrack * Macroinvertebrates	1	5.3	0.03 *
E. juncea				
Total plant	Wrack	1	0.1	0.76
	Macroinvertebrates	1	1.3	0.26
	Wrack * Macroinvertebrates	1	0.2	0.70
Total shoot	Wrack	1	0.7	0.42
	Macroinvertebrates	1	3.2	0.08 °
	Wrack * Macroinvertebrates	1	0.1	0.84
Roots	Wrack	1	0.1	0.86
	Macroinvertebrates	1	0.0	1.00
	Wrack * Macroinvertebrates	1	0.5	0.48

Table A5.5. Overview of the two-way ANOVA results on the effect of wrack burial (Wrack) and macroinvertebrate presence or absence (Macroinvertebrates) on P content, for two species (*Cakile maritima* and *Elytrigia juncea*) and plant organs separately. An asterix indicates a significant p-value (<0.05).

		df	*F*	*p*
C. maritima				
Total plant	Wrack	1	25.6	<0.001 *
	Macroinvertebrates	1	0.2	0.66
	Wrack * Macroinvertebrates	1	0.8	0.37
Total shoot	Wrack	1	18.3	<0.001 *
	Macroinvertebrates	1	1.3	0.26
	Wrack * Macroinvertebrates	1	1.7	0.20
Roots	Wrack	1	20.5	<0.001 *
	Macroinvertebrates	1	0.8	0.37
	Wrack * Macroinvertebrates	1	0.7	0.41
E. juncea				
Total plant	Wrack	1	0.5	0.48
	Macroinvertebrates	1	0.8	0.38
	Wrack * Macroinvertebrates	1	0.1	0.72
Total shoot	Wrack	1	0.0	0.97
	Macroinvertebrates	1	2.1	0.16
	Wrack * Macroinvertebrates	1	0.4	0.54
Roots	Wrack	1	2.1	0.16
	Macroinvertebrates	1	0.4	0.53
	Wrack * Macroinvertebrates	1	0.2	0.65

Table A5.6. Overview of the two-way ANOVA results on the effect of wrack burial (Wrack) and macroinvertebrate presence or absence (Macroinvertebrates) on N/P ratio, for two species (*Cakile maritima* and *Elytrigia juncea*) and plant organs separately. An asterix indicates a significant p-value (<0.05), while an open circle indicates a trend (p<0.10).

		df	*F*	*p*
C. maritima				
Total plant	Wrack	1	3.0	0.10 °
	Macroinvertebrates	1	0.7	0.41
	Wrack * Macroinvertebrates	1	5.7	0.02 *
Total shoot	Wrack	1	4.6	0.04 *
	Macroinvertebrates	1	1.1	0.30
	Wrack * Macroinvertebrates	1	5.7	0.02 *
Roots	Wrack	1	0.0	0.84
	Macroinvertebrates	1	0.7	0.40
	Wrack * Macroinvertebrates	1	0.5	0.48
E. juncea				
Total plant	Wrack	1	0.1	0.81
	Macroinvertebrates	1	0.5	0.48
	Wrack * Macroinvertebrates	1	1.2	0.28
Total shoot	Wrack	1	1.6	0.21
	Macroinvertebrates	1	1.0	0.34
	Wrack * Macroinvertebrates	1	1.5	0.23
Roots	Wrack	1	1.5	0.23
	Macroinvertebrates	1	0.4	0.56
	Wrack * Macroinvertebrates	1	1.2	0.29