Food web assembly along salt marsh succession:
A stable isotope approach

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INTRODUCTION

A core issue in community ecology is to understand the organization of a community.

In order to gain more understanding about the current community functioning, it is valuable to know how a community was build up over time. By studying the succession of a total ecosystem, this can be achieved.

Definition and visibility of succession

Along succession, an ecosystem changes from a pioneer stage towards a climax stage. Succession is a commonly accepted fundamental concept, during which natural, more or less predictable and orderly changes occur in the species composition and food web structure of a community over time (Odum 1969; Margalef 1997; Chapman and Reiss 1999 chapter 16; Pandolfi, Sven Erik et al. 2008).

Since succession occurs along time, the sequence of succession is usually invisible and therefore hard to study (Chapman and Reiss 1999). It would take ages to collect sufficient data. However, without long-lasting effort, succession can be studied in a few ecosystems, because the chronosequence of succession is visible along a spatial gradient (see box I).

BOX I: Primary versus Secondary succession
A chronosequence of succession is visible if different succession stages are visible in an ecosystem along space, instead of along time. Succession can be initiated by formation of a totally new ecozone with a primitive substrate with marginal nutrients (primary succession) or by disturbance of an already existing ecosystem (secondary succession). In order to understand the overall change where an ecosystem goes through, a study of primary succession is way more valuable since it start with really 'nothing'. Whereas, relatively a lot of ecosystems show secondary succession, visibility of the chronosequence of primary succession is really rare (Pandolfi, Sven Erik et al. 2008). One of the very few ecosystems where the chronosequence of primary succession may be visible are in some regressed glacier areas (Arctic and Alpines e.g. Rotmoos), some volcano erupted areas(e.g. Krakatau or the Hawaiian islands) and some salt marshes ecosystems (e.g. on the eastward extending island of Schiermonnikoog, The Netherlands) (Whittaker, Bush et al. 1989; Hodkinson, Coulson et al. 1996; Olff, De Leeuw et al. 1997; van Wijnen and Bakker 1997; Kaufmann 2001; Kaufmann, Fuchs et al. 2002; Hodkinson, Coulson et al. 2004; Raffl, Mallaun et al. 2006). Research in these ecosystems mainly focused on vegetation succession, while there live other organisms than plants also. Though it should be mentioned that along chronosequence of primary succession in glacial areas in the Arctic and Alpines - beside the investigation of the soil and the plant community - the invertebrate community was (although separately) investigated (Hodkinson, Coulson et al. 1996; Kaufmann 2001; Kaufmann, Fuchs et al. 2002; Hodkinson, Coulson et al. 2004; Raffl, Mallaun et al. 2006). Furthermore several studies on Schiermonnikoog research the influence of hare and geese on succession (but see box II).

Succession driven by bottom-up regulation

Present-day, we gained a noteworthy insight in the process of succession; especially the bottom-up regulation - driven by the vegetation - has been studied thoroughly.

The study of vegetation succession resulted in reliable knowledge about how plant species composition change over time (Tilman 1982; Whittaker, Bush et al. 1989; Olff, De Leeuw et al. 1997; van Wijnen and Bakker 1997; Raffl, Mallaun et al. 2006; Pandolfi, Sven Erik et al. 2008). That is, a change from r-strategy pioneer plant species that grow fast, towards K-strategy climax plant species that are better long-term competitors (Tilman 1986) (see also box III). Due to accumulating nutrients (especially nitrogen) in the soil along succession, primary producers increase, which results in an increasing primary biomass along succession (Olff, De Leeuw et al. 1997; van Wijnen and Bakker 1997).

Bottom-up regulation by the vegetation has long been thought to be the main drive of succession. Plants (primary producers) are consumed by herbivores (primary consumers) which are subsequently consumed by carnivores (secondary consumers) (Clements 1916). Following this, primary producers are the base of an ecosystem and they are therefore important for the succession of a total ecosystem.

BOX II: Soil accretation & plant composition and herbivory on the salt marsh of Schiermonnikoog

A unique ecosystem The salt marsh on the island of Schiermonnikoog is a unique ecosystem, because of at least two reasons.

Firstly, the chronosequence of primary succession is visible. The island gradually extents eastwards, consequently new ecozones are continuously developed. When moving from the eastern to the western part of the salt marsh on Schiermonnikoog - so from the young salt marsh towards the old salt marsh -, the chronosequence of succession is visible (Olff, De Leeuw et al. 1997; van Wijnen and Bakker 1997).

Secondly, an interaction between two ecosystems occurs. Approximately 20 times a year - during spring tide - the whole salt marsh is flooded by the adjacent Wadden Sea. Marine organic matter is left behind and sets the organisms on the salt marsh for a choice; they can either feed on terrestrial organic matter, on marine organic matter or on a combination of both sources. Beside marine organisms may influence the salt marsh too, while roosting on the salt marsh during high tide. In short, the terrestrial salt marsh ecosystem interacts with the marine Wadden Sea ecosystem and is thought to exchanges sources.

From the early 90's onwards, the primary succession has been studied here with a main-focus on soil, plants and herbivory.

Soil Van Wijnen and Bakker (1997) and Olff et al (1997) were the first to describe soil accretation, nitrogen accumulation and a change of plant species composition along the primary successional gradient on the salt marsh of Schiermonnikoog.

The pioneer stage starts here with nitrogen-limited bare sand, whereupon nitrogen-rich silty clay is deposited during floods (Olff, De Leeuw et al. 1997; van Wijnen and Bakker 1997). Besides this marine input, terrestrial organic matter increases too in the course of succession.

Marine organic matter Although the young (terrestrial) salt marsh is poor in inorganic nitrogen, it may be facilitated by the adjacent marine organic matter of the Wadden Sea ecosystem during a floods. Note that - besides the young salt marsh - the old salt marsh is flooded too, but the reliance of marine organic matter is thought to become weaker or disappear entirely along succession.

Plants Plants on the salt marsh are nitrogen limited and since the nitrogen content in the soil increases, plant productivity will increase along succession. Moreover, the salt tolerance of plants is thought to increase as well (van Wijnen and Bakker 1997). This causes a plant composition change along the succession gradient, from small halophytic plants with a r-strategy on the pioneer salt marsh towards big plants with a K-strategy on the climax salt marsh.

In the early pioneer stage *Salicornia europea* dominates, after which *Limonium vulgare* and also some *Puccinellia maritima* appears too. After 25 years of succession *Atriplex portulacoides* dominates still together with *Limonium* and *Salicornia* and a bit of *Puccinellia maritima.* Then *Puccinellia maritima blooms*. In the intermediate succession stage after 45-55 years *Festuca rubra* and *Artemisia maritima* take over. Subsequently *Elytrigia athericus* dominates the 100 year old sucession stage, which has been considered as a climax stage (Olff, De Leeuw et al. 1997; van Wijnen and Bakker 1997; Schrama unpublished). Though, recently it has been suggested that *Elytrigia* may not be the climax stage. A swampy vegetation might follow up, after which a reed vegetation might appear (personal communication with Han Olff and Maarten Schrama).

Herbivores From a certain stage onwards, herbivores appear. On our studied gradient (sand base elevation at 120 NAP) occur: domestic livestock (only during summer and in part of the 100 year stage), two geese species (partly migrants, stop-over-time: November-May), hares, and several invertebrate and microbial herbivore species (such as nematodes).

Both, the density of geese and hare, peak around the intermediate age and productivity stage (though slight variation between studies does exist: Olff, De Leeuw et al. 1997; Olff and Ritchie 1998; van der Wal 1998; van Wijnen, van der Wal et al. 1999; Kuijper 2004; van der Graaf 2006). The following species are preferred: Brent geese: *Puccinellia maritima* but also marine plants (Olff, De Leeuw et al. 1997; van Wijnen, van der Wal et al. 1999) / Barnacle geese: *Puccinellia maritime* (& *Festuca rubra*) (Smit and Wolff 1980) / Hares: *Festuca rubra*, (Kuijper 2004).

Herbivores influence succession Firstly, during winter, hares set back succession for >25 years by delaying the bushy *Atriplex portulacoides* encroachment and thereby facilitating brent geese. By feeding on *Atriplex*, hares create space for the earlier succession plant *Puccinellia,maritima* on which brent geese forage (van der Wal 1998). Secondly, herbivory suppresses the mineralization and keeps thereby the nutrient availability for plants low (vandeKoppel, Huisman et al.

However primary producers are not the only driving force of succession in an ecosystem. After all, consumers do also influence primary producers as well as other consumers. Top-down regulation may therefore also play an important role in driving an ecosystem's succession too.

Succession driven by top-down regulation by higher trophic organisms

Higher trophic organisms (herbivores, carnivores and detritivores) influence an ecosystem by top-down control. Several articles report the impact and change of this top-down regulation of higher trophic organisms on an ecosystem along succession.

Firstly, large and intermediate sized herbivores are reported to delay vegetation succession and to facilitate other organisms (see box III). Invertebrate herbivores are reported to influence plant succession too (Brown and Gange 1992). On the other hand, the expected regulating effect of herbivorous nematodes on vegetation was marginal (p.59, van der Wal 1998) (see box III). Generally speaking, though, it may be clear that herbivores do influence succession.

Secondly, detritivores consume dead producers and consumers, so they influence the ecosystem too. Wardle et al. (1995) studied the food web and trophic structure of small detritivorous invertebrates during primary succession in sawdust. Along succession, the food chain length and food web complexity first increased after which they became stable. Considering all this, detritivores may form an important determinant for the successional development of an ecosystem.

Thirdly, invertebrate community assembly - in general - changes along succession. In an Arctic ecosystem, invertebrate species composition changes and species richness increases along succession (Hodkinson, Coulson et al. 2004) (see box IV). The microbial soil communities have also been reported to change along succession and might have feedback interactions with, for instance, plant roots (Mahaming, Mills et al. 2009). A study towards soil macro-invertebrates in grasslands in the Drenthse Aa, The Netherlands, showed a strong correlation effect between soil fauna and plant species composition. Moreover these soil macro-invertebrates are thought to enhance succession and plant species diversity. By selectively suppressing dominant early succession plant species, soil fauna enhances later successional plant species as well as subordinate early successional plant species (De Deyn, Raaijmakers et al. 2003). So not only the plant species composition changes along succession, but higher trophic species composition does too.

Finally, an ecosystem-wide salt marsh study in Plum Island Estuary, Massachusetts showed that the community is influenced by bottom-up and top-down regulation concurrently (Johnson, Fleeger et al. 2009). Although Johnson and Fleeger did not research the top-down and bottom-up regulation as driving forces of succession, there results seem to bear relevance to the topic of succession. Duffy et al. (2007) even take it further and describe that the higher trophic organisms - which show a top-down regulation - are found to have a stronger influence on the diversity of an ecosystem, compared to lower trophic levels that have a bottom-up regulation.

What drives succession: bottom-up and/or top-down regulation

All abovementioned studies were of great value for the understanding that ecosystem succession is not only controlled by bottom-up regulation – as described by Clements (1916) - but also by top-down regulation by higher trophic organisms. Probably both regulation mechanisms are important for the process of succession.

However, these abovementioned studies only report how a single species or small group of organisms change and influence an ecosystem (along succession), while lacking information about the changes where a total community goes through along succession.

The driving force of succession can only be understood, when including the changes and influences of a total community in its ecosystem along succession, that is including all species from all trophic levels.

Towards a modern concept of succession

Many theories have been postulated about the changes and drive of an ecosystem along succession.

Odum (1969) attempted to give an overview of the - mostly theoretically based - assumptions that have been made about succession. Also Margalef postulated a theory of succession. For a brief summary of Odum's overview and Margalef's ideas, see box I.

About 10 years after Odum's overview, **Oksanen** (1981) described that a simple food chain (primary producers - primary consumers - secondary consumers) would be developed along succession.

BOX III: CONCEPT OF SUCCESSION (Odum & Margalef)

According to Odum's overview about the concept of succession (see for a complete overview, table 1, Odum 1969): The total **biomass** of an ecosystem will increase continuously in the developmental stage of succession and the ratio of primary production is higher than the community respiration (P/R-ratio exceeds 1). Whereas - once the climax stage has been reached - the total biomass is thought to stabilize at a high equilibrium and the **P/R-ratio** would approach 1(Odum 1969).

The linear plant-herbivore-carnivore **food chain** which occurs in the pioneer succession is thought to develop into a complex detrivoreous-pathway-controlled **food web** in the climax succession.

Along succession; **species diversity** and number of individuals are thought to increase (Odum 1969). Furthermore, the system would direct towards homeostasis in which internal **symbiosis** would develop(Odum 1969).

Finally, the **nutrient cycle is** thought to change along succession from external towards internal fuelled. The nutrient cycle in the pioneer stage is open, which means that the ecosystem is partly dependent on at least one adjacent ecosystem. In the course of succession towards the climax stage, the ecosystem will close its nutrient cycle and the nutrient exchange rate within the ecosystem will slow down(Odum 1969; Pandolfi, Sven Erik et al. 2008).

Margalef compared succession of an ecosystem, with the life of an organism: from simple and bare, towards a complex and full ecosystem, after which the ecosystem would get into decay. During this decay, for instance, **species richness** and the **number of trophic levels** (which Margalef assumed to differ along succession with a maximum of up to 5 chains) would shrink again (Margalef 1997).

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Although the concept of succession ((Odum 1969; Oksanen 1981; Margalef 1997) is generally accepted and widely used in scientific articles (entering the term 'succession' at ISI_Web_of_Science shows 30.758 hits), it mostly relies on theoretically based assumptions and on bottom-up regulation based argumentation, which for long was seen as the only regulating mechanism of succession. No single article ever reported yet a fundamental empirical study about how the food web composition, complexity and trophic structure in a total ecosystem behaves along succession, that is, including all species. This lack in fundamental empirical research of successional changes in a total ecosystem was already perceived by Odum in 1969.

The general accepted theory of succession, might therefore not hold for a total community and even be (partly) untrue. Organisms are limited by the amount of fixed energy in an ecosystem (Hairston, Smith et al. 1960). Following this, Oksanen (1981) was probably right when describing the linear 'plant-herbivore-carnivore' food chain build-up along succession. Though, if Odum (1969) was correct that a pioneer ecosystem interacts with other ecosystems and derives organic matter from this external source (as visualized in Figure 1) , than detritivores may feed on these external sources and subsequently carnivores might feed on these detritivores. This would mean that - without existence of internally produced plants or herbivores - detritivores and carnivores can be present in a pioneer stage. With that, contrary to the assumptions: 1) it might be the detritivores – instead of the autotrophic primary producers–who colonize a new ecozone first, 2) the change from a linear food chain towards a complex food web (Odum 1969) might be incorrect and 3) the thought of Margalef (1997) that number of trophic levels will gain along succession, may thereby be incorrect too.

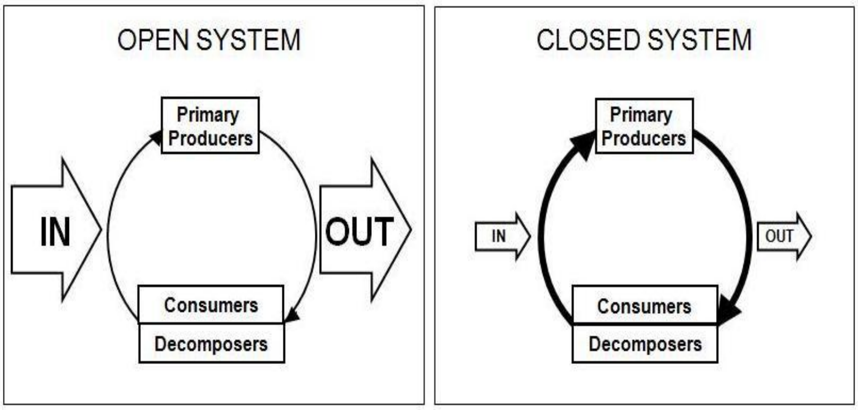


Figure 1: Along succession, ecosystem change from an externally fuelled system (open system) towards an internally fuelled system (closed system).
NB: The internal cycle is stronger in a closed system.

With the current knowledge that top-down regulation is probably also important in driving succession, the question rises whether or not the - almost for a whole century assumed - concept of succession rightfully accepted.

Food web complexity and trophic structure of a total ecosystem and its interactions with other ecosystems therefore deserves to be studies empirically.

This research

In this study, the changes of the total ecosystem - from soil to top-predators - along the chronosequence of the primary succession on the salt marsh on the island of Schiermonnikoog was studied.

The salt marsh on the island of Schiermonnikoog, where the chronosequence of primary succession is visible, is a unique ecosystem (see box III). In particular the soil composition, plant community and herbivory have been well-studied here along succession (Olff, De Leeuw et al. 1997; van Wijnen and Bakker 1997; Olff and Ritchie 1998; van der Wal, van Lieshout et al. 2000; Kuijper 2004). See for details box III. Recently Schrama did investigate the invertebrate abundance and species composition along succession (see Appendix).

This report presents the abundance and species composition of all mammal and bird species along succession. With that, the abundance of all species in a total ecosystem along succession are investigated.

The actual aim of this study was to gain more insight in the basic changes where a total ecosystem goes through over time. We tested two main research questions. Both research questions and hypotheses are shown in Table 1.

RESEARCH QUESTIONS & HYPOTHESES:	
1. How does a food web assemble along succession?	
Food web complexity	Development from a simple food chain (plant-herbivore-carnivore) towards a complex and detritivorous-pathway-controlled food web.
Trophic structure	The number of trophic levels will increase (up to 5) along succession
Food web regulation	Food web assembly is regulated by bottom-up control & top-down control.
2. Does the salt marsh ecosystem changes from an open towards a closed regulated ecosystem?	
Nutrient cycle	The open nutrient cycle in the pioneer stage will gradually close its cycle along succession, which means that the ecosystem develops to a self-sustaining climax stages which needs no external source input anymore. >> The interaction between the terrestrial salt marsh and the marine Wadden sea ecosystem will weaken along succession

Table 1: Both research questions with corresponding hypotheses

The changes of the total community of the salt marsh ecosystem on the island of Schiermonnikoog were studied along succession, by doing field observations as well as stable isotope analyses.

Field observations and measurements were accomplished in order to get a reliable overview of the abundance per species group, the food web complexity and to gain more insight in possible species interactions along succession.

A combination of stable nitrogen and carbon isotope analysis has proved to be valuable in determining, respectively, the trophic structures and the community's sources (Peterson, Howarth et al. 1985; Hobson and Welch 1992; Post 2002; Stapp and Polis 2003; Abrantes and Sheaves 2008; Page, Reed et al. 2008; Choy, Richard et al. 2009).

Stable Nitrogen isotope analyses were performed to get insight in the food web assembly along succession, while stable C isotope analyses were performed to show the degree of interactions between the terrestrial salt marsh ecosystem and the adjacent marine Wadden sea ecosystem.

MATERIAL & METHODS

The change in trophic structure, food web composition and -complexity along the successional chronosequence of the salt marsh of the Dutch Wadden islands, Schiermonnikoog (53°30'N, 6°10'E), was studied between 10 March 2010 and 30 May 2010.

In order to gain in-depth knowledge about how a food web assembles along succession, two main measurements had to be accomplished.

Firstly, the species occurrence and density along the succession gradients had to be analyzed by field observations.

Secondly, the trophic structure, food web complexity (who eats whom) and the interaction between the two ecosystems (salt marsh and Wadden Sea) was analyzed by, respectively, stable δN - and δC-isotope analyses.

Succession gradient

Along the successional chronosequence, 7 succession stages were selected. The seventh succession stage (100 yrs) is partly ungrazed and partly grazed by cattle; here a plot for both situations was selected. This resulted in a total of 8 plots over the total succession gradient.

Figure 2: Location of succession stages on the salt marsh of the island of Schiermonnikoog (53°30'N, 6°10'E), The Netherlands.

The age of the succession stages was determined by the clay thickness. Whereas the base elevation of the salt marsh of Schiermonnikoog remains constant along the succession gradient - 120 cm above Dutch Ordnance Level (NAP)-, the clay layer thickness increases over time (van Wijnen and Bakker 1997). The clay layer thickness therefore represents the degree of succession.

The ages of the succession stages were estimated at 0, 10, 25, 35, 45, 55 and 100 years old, corresponding with clay layer thicknesses between 0 and 15 cm. See for the position of these succession stages in Figure 2.

SPECIES ABUNDANCE OF HIGHER TROPHIC LEVELS

All species that live at each of the eight plots along the selected succession gradient were quantified, using different trapping techniques. The species occurrence and density of all plant, invertebrate, hare and goose species were already investigated in May 2009 (personal communication with Maarten Schrama), whereas mouse and bird species - others then goose – had to be quantified in March-April 2010.

Bird observations

Within each of the seven succession stages, a 50*100m plot was selected in which birds were repeatedly observed by using a pair of binoculars (Bynolyt Tern, 8x45) and a telescope (Swarovski, 60x).

Each observation endured 30 minutes and was preceded by a period of 5 minutes after arrival, to reestablish the animal's natural behavior. While observing, the observer always kept a distance of 50-200m from the plot. At departure the observer walked through the plot, to check for possible underestimations of the amount of birds in a plot.

Per species, the time a certain number of individuals visited a plot was noted. Here a difference between birds in the air and on the ground was made, which was again split up into foraging- and nonforaging birds. Furthermore weather conditions (sun, wind and rain), tidal situation and 'days since the total salt marsh was flooded' were taken into account. In total 5 visits to every location were made in the period of March-April 2010. To standardize different bird observations, we represent bird count in bird minutes. This which was calculated by taking the 'cumulative time for each of the species' times 'the number of birds present'.

Raptor pellet determination

In order to gain insight about the raptor's food resource, pellet contents were identified.

The pellets were collected on the roosts. The roosts of raptors were traced around dusk – or in case of the short-eared owl during the day. In some cases fresh pellets under a pole, where a raptor was just spotted, were identified too.

The content of the pellets were examined by using identification guides (van Diepenbeek 1999; VZZ 2001). The pellet always contained mice or hare hair and/or small bird feathers, which was noted as mice sp., bird sp. or hare. In case a pellet contained a mice skull, the exact species could be identified and here the exact mice species was noted instead of only mice sp.

Mice occurrence

In a strip of 2x100m within each of the 8 plots, the number of mice holes were counted, mapped and filled up with a dot of hay. Every 2-15 days, the numbers of reopened mice holes per strip were counted, new mice holes were noted and all holes were refilled with hay. The strips were checked repeatedly during the period between 16 March and 12 April 2010.

Where the number of mice holes only indicates that there has once been mice activity, the number of reopened mice holes also gives an indication of recent mice activity (van Diepenbeek 1999).

Although it is not known how many mice are present per mice hole, this mice-hole-method can be used relatively, as a comparative analysis of mice activity over succession (Romankow-Zmudowska 1996; Lange, Twisk et al. 2003).

FOOD WEB ASSEMBLY & ECOSYSTEM INTERACTION: STABLE ISOTOPE ANALYSIS

In order to gain more knowledge about food web assembly and ecosystem interaction along succession, stable carbon and nitrogen isotope analyses were accomplished. A low stable carbon ($\delta^{13}C$) isotope value represents a terrestrial signal, while a high $\delta^{13}C$ value represents a marine signal (Park and Epstein 1961; Fry and Sherr 1984).

From all encountered species along the succession gradient, the species which represented a certain species group and/or which occur along most or all succession stages were sampled. Furthermore samples were taken from the marine and terrestrial sources along succession.

The isotopic values of all primary producers (marine and terrestrial), terrestrial dead organic matter

Sample	Tissue	Collection period	Measured stages
Terrestrial soil (SOM)	Total	27 & 28 April	10, 45, 100[a&b] yrs
Terrestrial dead plants (TOM)	Total	27 & 28 April	10, 45, 100[a&b] yrs
Marine organic particles (POM)	Deposit	23 – 27 April	10, 45, 100[a&b] yrs
Diatoms	Total	23 – 27 April	10, 45, 100[a&b] yrs
Fucus	Leaf	23– 27 April	10, 45, 100[a&b] yrs
Plants	Young leaf	19– 27 April	10, 45, 100[a&b] yrs
Detritivorous invertebrates	Total	19– 27 April	0-100[a&b] yrs
Herbivorous invertebrates	Total	8-10 May	0-100[a&b] yrs
Carnivorous invertebrates	Total	8-10 May	0-100[a&b] yrs
Hares & common voles	Hair	10 March - 15 April	
Hares (& common voles)	Dropping	Unsucceeded [c]	10, 45, 100[a&b] yrs
Raptors	Pellet	8 March – 13 April	55 yrs
Raptors	Feather	18 March	55 yrs
Invertebrate-feeding bird: Wheatear	Feather	20 March	100 yrs
Marine bird: Lesser Black-backed gull	Dropping	20 May	35 yrs

Table 2: Collected samples of which the δN - and δC-ratios along the succession gradients were measured.

and soil were only measured in the succession stages of 10, 45 and 100 years old, because it was expected that these samples wouldn't change in nitrogen and carbon value. The carbon and nitrogen isotopic values from all other species samples were measured in all 8 succession stages.
For an overview of the period and stages of all collected species, see Table 2.

Collection of sources (terrestrial & marine organic matter)

Terrestrial plants

8 plant species were - in case present - collected all succession stages (see for details Appendix I). Since most animals tend to forage on the young part of plants, at least 5 young leaves of preferably different plant individuals were collected. The collected plant species showed no visible intraspecific difference between succession stages, therefore stable isotope analysis were only accomplished for the concerning plant species in the stages of 10, 45 and 100 years after succession.

Terrestrial soil top-layer

Terrestrial soil samples were taken by mixing soil samples from the upper 0,5 cm that were taken from 5 different places in every 5x5 plot spaced 2 meters apart. Also here, stable isotope analysis were only accomplished in stage 10, 45 and 100 yrs.

Terrestrial dead plants

Terrestrial dead plants were sampled by collecting 5 subsamples of dead and half-degraded plants spaced 2 meters apart. Dead plants which were still in its former horizontal position were not taken. Also here, stable isotope analysis were only accomplished in stage 10, 45 and 100 yrs.

Marine particle organic matter

To collect marine organic matter, cups (Φ 10 cm) were buried into the ground to trap marine clay which deposits during a flood. However, due to extremely mild weather conditions, the salt marsh unfortunately was not flooded during our research period.
Therefore, marine organic matter was captured by manually filtering with a fine-darned filter net (diam. 0,5mm), along the coast in Wadden-Sea-connected creek estuaries as close as possible to each succession stage during high tide when the sea water was turbid enough to carry suspended matter. Also here, stable isotope analysis were only accomplished in stage 10, 45 and 100 yrs.

Diatoms (marine source)

Diatoms were collected on the mudflat near to each succession stage. To extract the diatoms from the silt, a layer of fine-darned filter gauze and a 2 mm layer of combusted sand were put on top of the diatom-containing silt. The construction was sprinkled with filtered sea water and exposed to (not too strong) daylight for about 6 hours. The diatoms migrate towards the light and since they are the only organisms which are capable in migrating through both layers, only the diatoms migrate on top of the combusted sand (invented by P. van Rijswijk, but see: Compton, Kentie et al. 2008). The combusted sand was rinsed in water and since sand sinks quicker as the lighter diatoms, the water with the diatoms could be extracted. Also here, stable isotope analysis were only accomplished in stage 10, 45 and 100 yrs.

Fucus sp. (marine source)

The brown algae genus *Fucus sp.* was collected near the coastal line as close to the succession stage. Per samples, at least 5 different individuals were used.

Collection of invertebrates

Invertebrates were collected with a D-vac machine with a strong sucking capacity, by hand or by using an insect net. The D-vac machine is known as an accurate and less-damaging to collect samples (Harper and Guynn 1998).

Detritivorous invertebrates were collected as soon as the first leafs arose. Herbivorous invertebrates were collected during the period that most young leaves arose. During this same period - when the herbivorous insects bloomed - the predatory invertebrates were collected.

In total 10 herbivore invertebrate species were collected of which the 3 snout beetle (*Elateridae*) species were grouped so that the total came on 8. In total 14 carnivorous invertebrate species were collected, of which all 5 money spiders (*Linyphiidae*) were grouped so that the total came on 9. In total 11 detritivorous invertebrate species were collected (see for details Appendix I).

Collection of birds and mammals

During the total field work period of March-May 2010 feather-, hair- and dropping samples of birds and small mammals (dead or alive) were collected. Since birds have a large home range and move freely along the succession gradient or even further, no differentiation in succession stages was made. Although hares have large home ranges too (28,7 ± 8,5 ha) (Kunst, van der Wal et al. 2001)), hair and dropping samples were sampled in 3 succession stages instead of 1. This was done to show possible small diet differences along the succession gradient.

Stable Nitrogen and Carbon isotope measurement

All samples were stored frozen and processed by freeze-drying and grinding with a pebble mill, before the stable δC- and δN- isotopes ratios could be measured. The isotope measurements were accomplished with the elemental analyzer-isotope ratio mass spectrometry machine (EA-IRMS, flash 2000.delta V.MS, Thermo) from the Royal Netherlands Institute for Sea Research (NIOZ).

DATA STORAGE & STATISTICS

All data were stored in Access (2003), statistical analyses and graphs were made by using Statistica and Sigmaplot.

Waypoint-tool and Google-Earth were used to map the raptors along the succession gradient of salt marsh, to assign the raptor observations subsequently to the closest succession stage.

The stable isotope values (‰) were calculated with the formula shown in Figure 3.

Gaussian distributions and exponential decay statistical analyses were use to check for significant patterns in the species abundance. General Linear Model ANOVA analyses were used to see if stable isotope values did change along succession.

$$\delta^{15}N\ (‰) = \frac{\left(\frac{^{15}N}{^{14}N}\right)_{sampled} - \left(\frac{^{15}N}{^{14}N}\right)_{standard}}{\left(\frac{^{15}N}{^{14}N}\right)_{standard}} * (1000\ ‰)$$

$$\delta^{13}C\ (‰) = \frac{\left(\frac{^{13}C}{^{12}C}\right)_{sampled} - \left(\frac{^{13}C}{^{12}C}\right)_{standard}}{\left(\frac{^{13}C}{^{12}C}\right)_{standard}} * (1000\ ‰)$$

Figure 3: Formula for the corrected stable Nitrogen and Carbon isotope value

RESULTS

SPECIES ABUNDANCE OF HIGHER TROPHIC LEVELS

Invertebrate-feeding birds along succession

In Figure 5 is visible that birds who forage on invertebrates, are most abundant in the intermediate stage of succession (Gaussian: R^2 = 0,575; t_α = 4,14; p_α = 0,014; t_β = 3,33; p_β = 0,029).

Note that the total number of bird minutes in stage 35 is much lower than might be expected in comparison with the other bars. Only jackdaws are abundant in the 35 years stage.

Small mammal activity along succession

From all - on the island occurring - three small mammals species, only common vole holes were found.

The total activity (total number of vole holes) along succession, significantly fit a normal distribution (Gaussian: R^2=0,904, t=4,730, p=0,009) (see Figure 4). On the other hand, the current activity (total number of reopened vole holes) along succession, does not significantly fit a normal distribution (Gaussian: R^2=0,729, t=2,111, p=0,102).

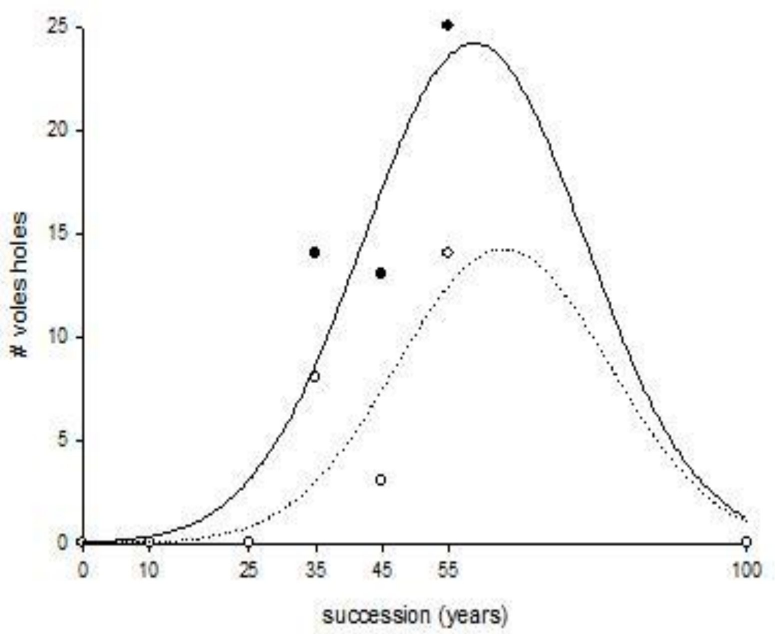


Figure 4: Common vole abundance along succession
Solid line: total activity (total number of found vole holes)
Dashed line: current activity (total number of reopened vole holes)

The highest mice activity was found in the stage of 55 years after succession (within 29 days, 25 mice holes were closed with a dot of hay of which 14 mice holes were reopened by mice).

All vole holes were found in the 35, 45 and 55 years old succession stages (see Figure 4). In the other succession stages of 0, 10, 25 and 100 years old, vole holes were only found in higher elevated salt marsh areas (>120 cm Dutch Ordinance Level).

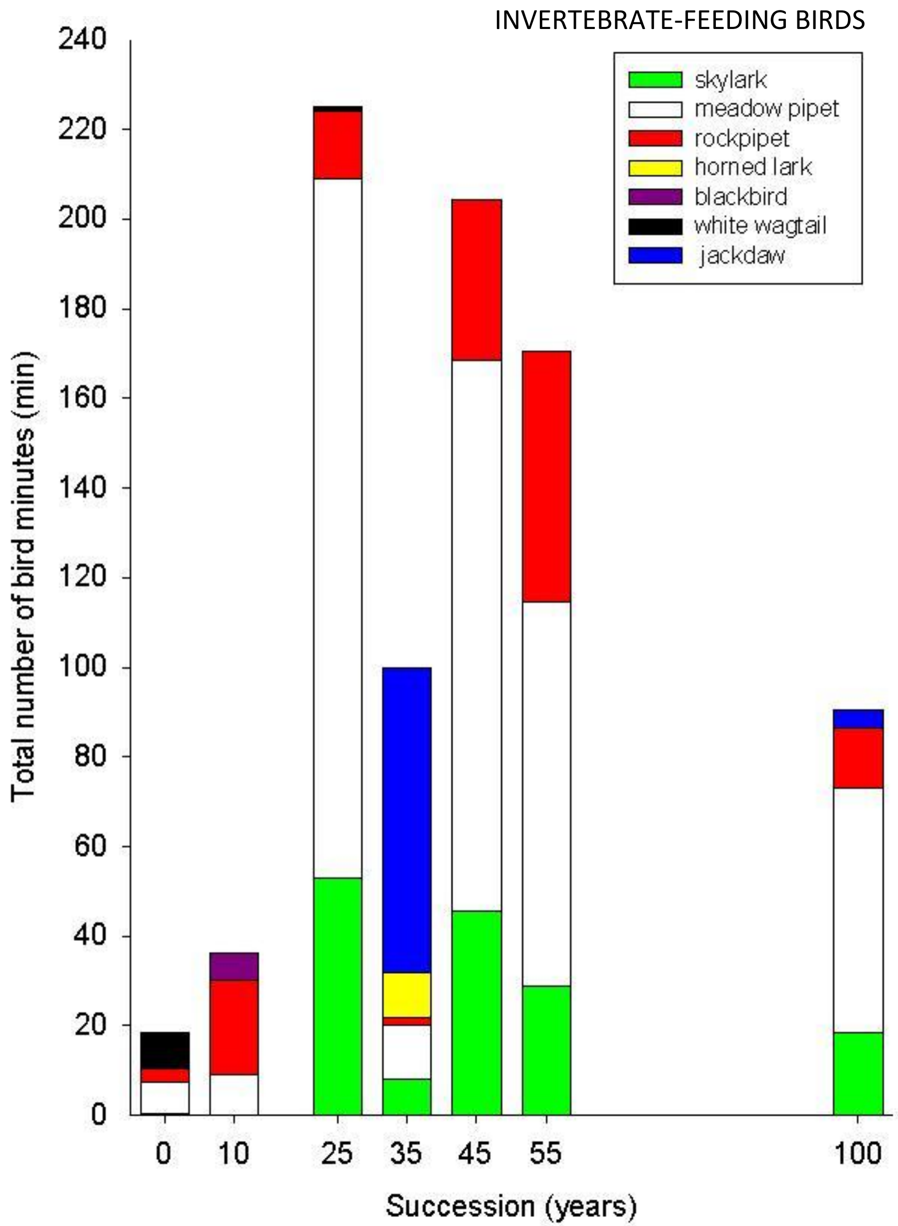


Figure 5: Invertebrate-feeding birds along succession

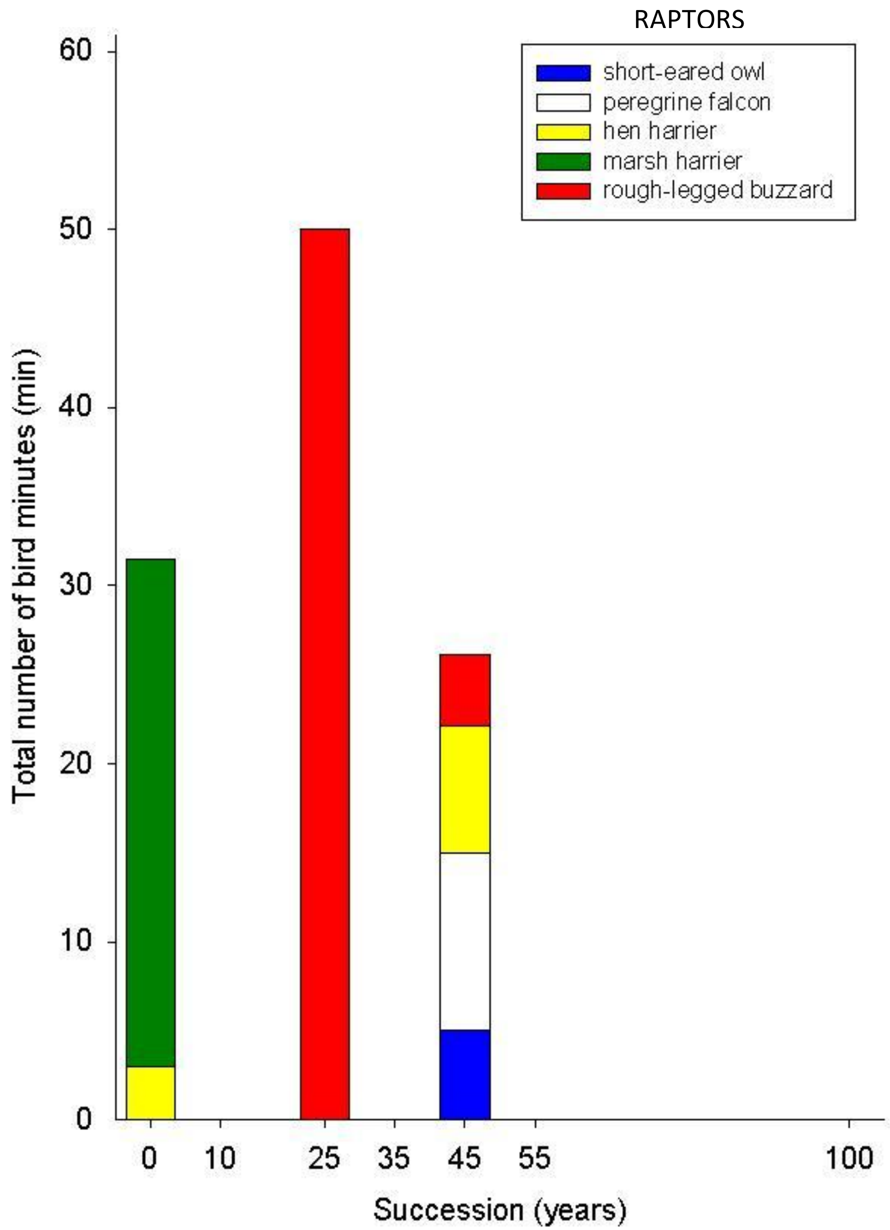


Figure 6: Raptors along succession

Raptors along succession

In Figure 6 is shown that raptors are active on our salt marsh succession gradient as well. No statistical trends in raptor abundance could be discovered. Only in the succession stage of 0, 25 and 45 years old raptors were seen while observing a stage. Nonetheless raptors of various species were certainly present in all other stages (personal observations adjecent to the 30-minute-observations).

Raptor diet analysis

The pellets - which were mostly found on the roosts - of various raptor species, did contain various prey which can be seen in .

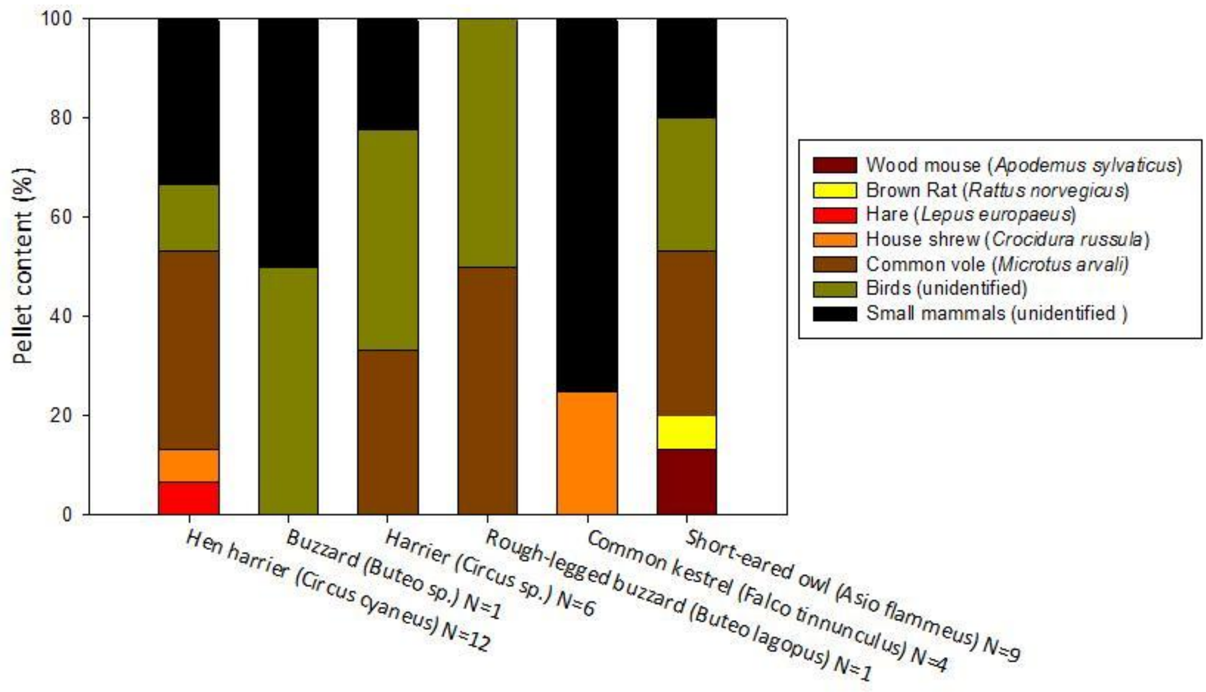


Figure 7: Raptor pellet content

During the day short-eared owls, who were mostly spotted near the 45 and 55 years old succession stages (extra personal observations), did have quite a varied diet which included all three mice species on the island.

Note that the small variance in diet of rough-legged buzzards and common buzzards are mainly due to an extremely small sample size.

Hen harriers were the only raptor species that fed on (young) hare.

Feral cats feed on hares

Many hare carcasses - which were munched by feral cats - were found all along the salt marsh, especially near dunes. Around these dunes and on the east-west directed small path which routs all along the salt marsh, many feral cat droppings were found. Furthermore, several old rabbit holes with hare carcasses and feral cat droppings in front of it were found. These holes are supposedly occupied by feral cats nowadays.

It seems that many cat droppings contained hare hair, but a substantial quantity of identifications has yet to be done.

Marine birds along succession

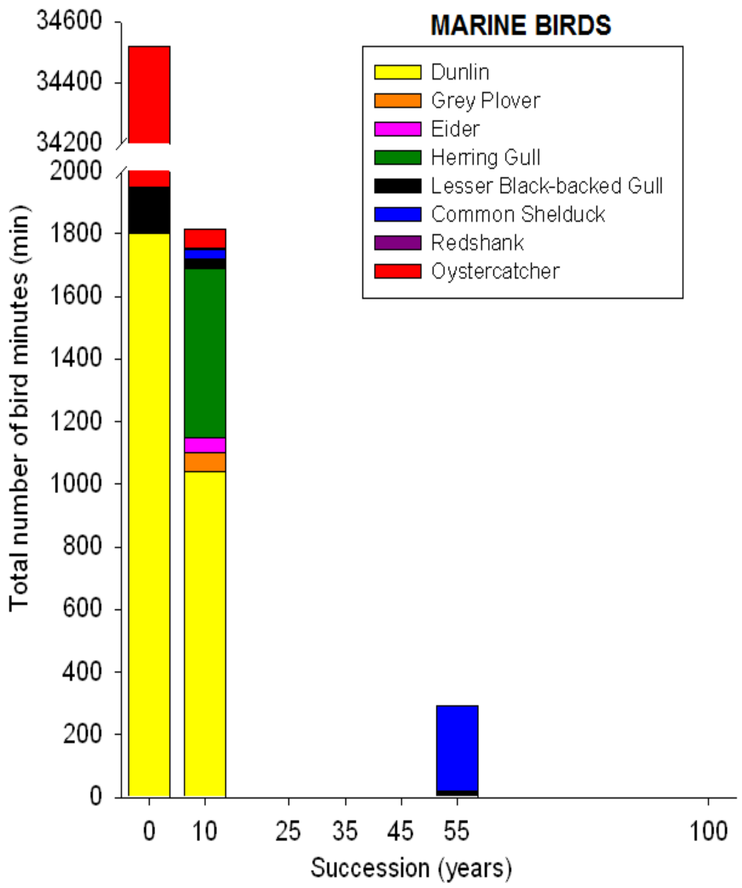


Figure 8: Marine birds along succession

As is shown in Figure 8 the abundance of marine birds along succession does follow an exponential decay (Exponential decay $_{\text{marine bird}}$: R^2= 0,999; n=7; t_α= 262,72; p_α= <0,0001; t_β= 40,62; p_β= <0,0001). Marine birds are extremely abundant in the climax stage of up to ten years of succession, this area is relatively close to or even on the flood mark of the Wadden Sea.

During high tide the stage up to 10 years of succession is used as roosting place for these marine birds.

Especially dunlins and oystercatchers were high abundant here. Only dunlins were observed to forage. Most of these marine birds were observed to excrete faeces (guano) while roosting.

The guano of these marine birds gives a marine signal (Lesser Black-backed Gull: $\delta^{13}C$ = -17,99(±1,45)) and may subsidize the salt marsh ecosystem with this extern guano source (see page 19 & 28).

FOOD WEB ASSEMBLY & ECOSYSTEM INTERACTION: STABLE ISOTOPE ANALYSIS

A visualization of the change in food web position along succession

The food web position (source input & trophic level) along the chronosequence of all seven succession stages are shown in Figure 9.

NB: 1) All money spiders (*Linyphiidae* sp.) were pooled together, as they did not significantly differ from each other(GLM Anova, corrected for change along succession: n=25, $F_{\delta N}$ = 2,07, $p_{\delta N}$ = 0,125, $F_{\delta C}$ = 1,77, $p_{\delta C}$ = 0,176). 2) Neither did the three snout beetle species (*Curculionidae sp.*) differ significantly (GLM Anova, corrected for change along succession: n=13, $F_{\delta N}$ = 20,58, $p_{\delta N}$ = 0,579, $F_{\delta C}$ = 2,81, $p_{\delta C}$ = 0,113) and were therefore pooled together. 3) In each graph in Figure 9a-9g reference lines for *Festuca rubra* - a species which is present in most succession stages along our gradient - are shown.

Food web position per category

As expected, terrestrial primary producers have a signal which is terrestrial (low $\delta^{13}C$) and low in trophic level (low $\delta^{15}N$), while marine primary producers - see e.g. stage 10 yrs in Figure 9 - have a marine signal (high $\delta^{13}C$). Furthermore, compared to terrestrial primary producers, marine primary producers give a significantly higher $\delta^{15}N$ signal (Table 3) which implies a higher trophic level.

Figure 9 clearly shows the difference in isotope signals between the categories (primary producers, herbivores, carnivores and detritivores). The trophic level of herbivores significantly differs from the terrestrial primary producers with - on average - an 2,12‰ increase in $\delta^{15}N$. Whereupon carnivores show on average an 2,57 ‰ increase in trophic level compared with herbivores.

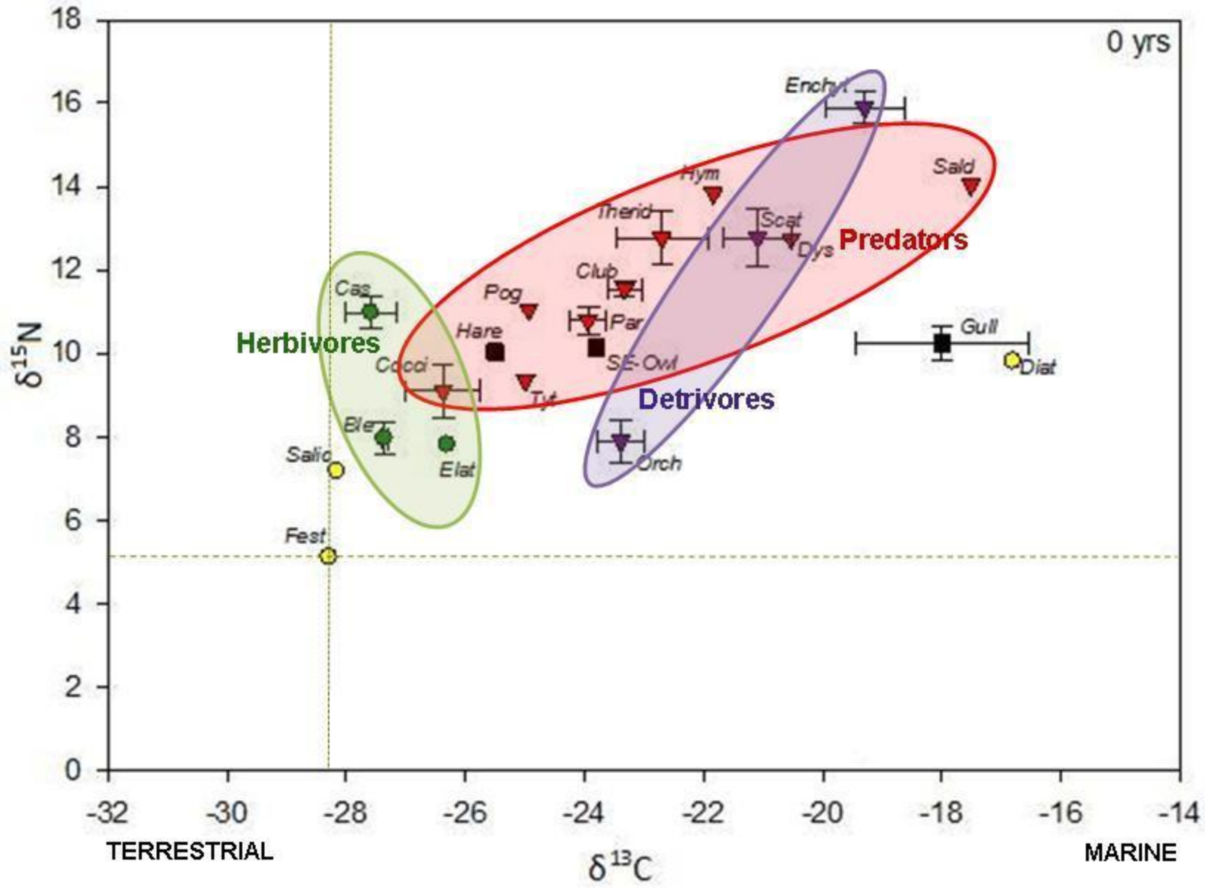
Remarkable extremes & outliers

Spartina anglica, a endophytic C4-plant which occurs in pioneer succession stages (see Figure 9, stage 10yrs), gives a high signal in both isotopic components. *Spartina anglica* is thereby substantially different from the other terrestrial primary producers.

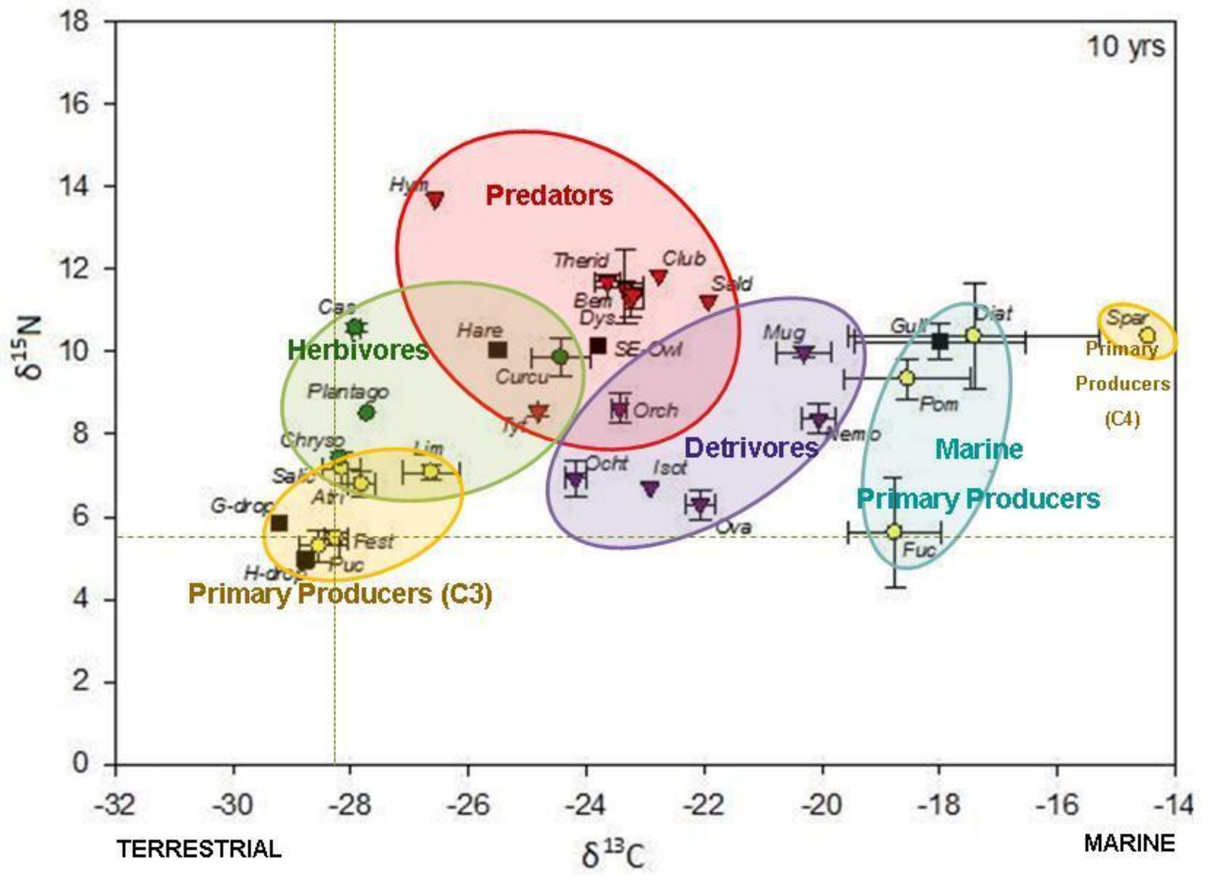
				$\delta^{13}C$		$\delta^{15}N$	
category	n	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	F	p	F	p
Ter prim prod Mar prim prod	15 6	-27,36 -17,81	6,34 8,10	40,05	**<0,001**	5,34	**0,033**
Ter prim prod Herbivores	15 22	-27,36 -26,87	6,34 8,46	0,09	0,773	9,28	**0,004**
Herbivores Carnivores	22 56	-26,87 -24,50	8,46 11,03	37,02	**<0,001**	42,30	**<0,001**
Herbivores Detritivores	22 35	-26,87 -23,30	8,46 7,30	89,19	**<0,001**	2,00	0,163
Carnivores Detritivores	56 35	-24,50 -23,30	11,03 7,30	16,43	**<0,001**	83,53	**<0,001**

Table 3: Difference in stable isotope values between categories

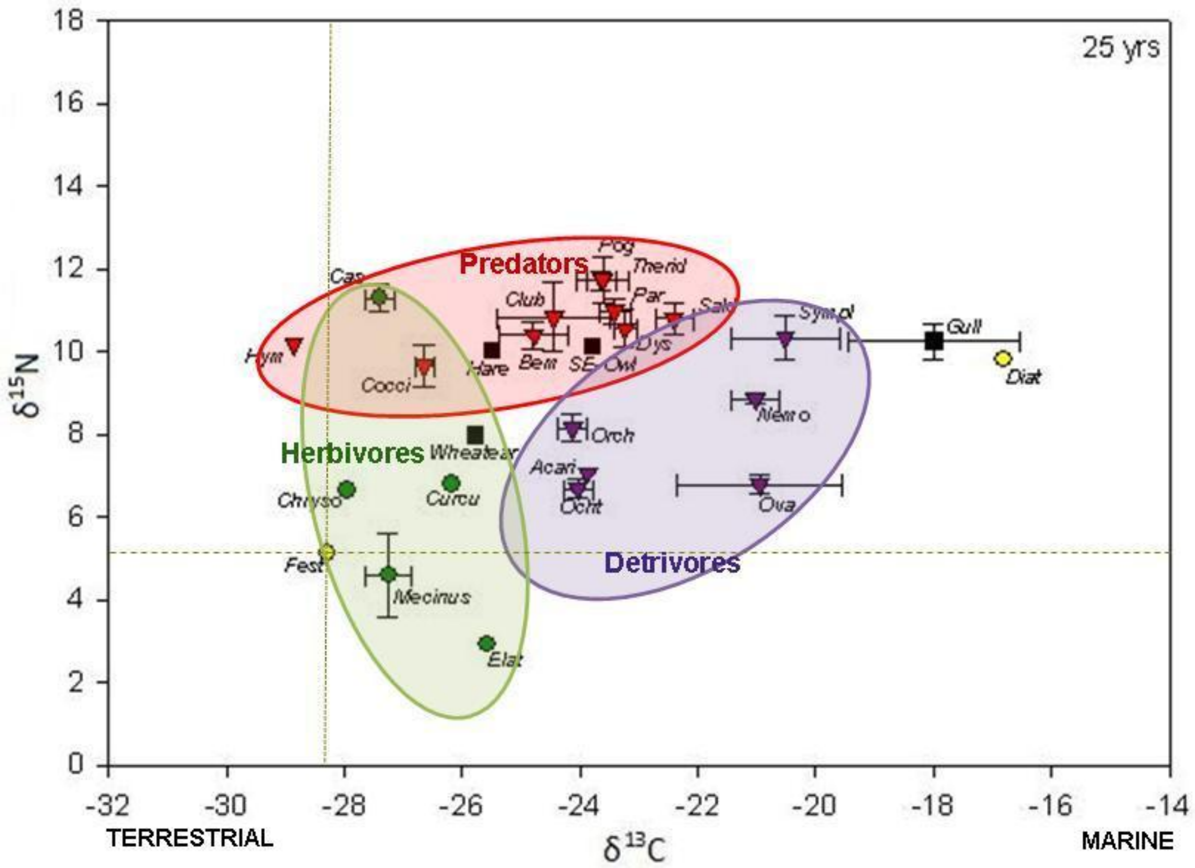
Potworms, *Enchytraea* spp. (Enchyt), occur in the youngest succession stage (0 yrs). They have an extremely high $\delta^{15}N$ value; higher than every other measured organism on the salt marsh (see Figure 9, stage 0 yrs). On the other hand, the *Isotoma riparia* springtails, which occur in stage 45, 55 and 100 yrs, do have an extremely low $\delta^{15}N$ value.

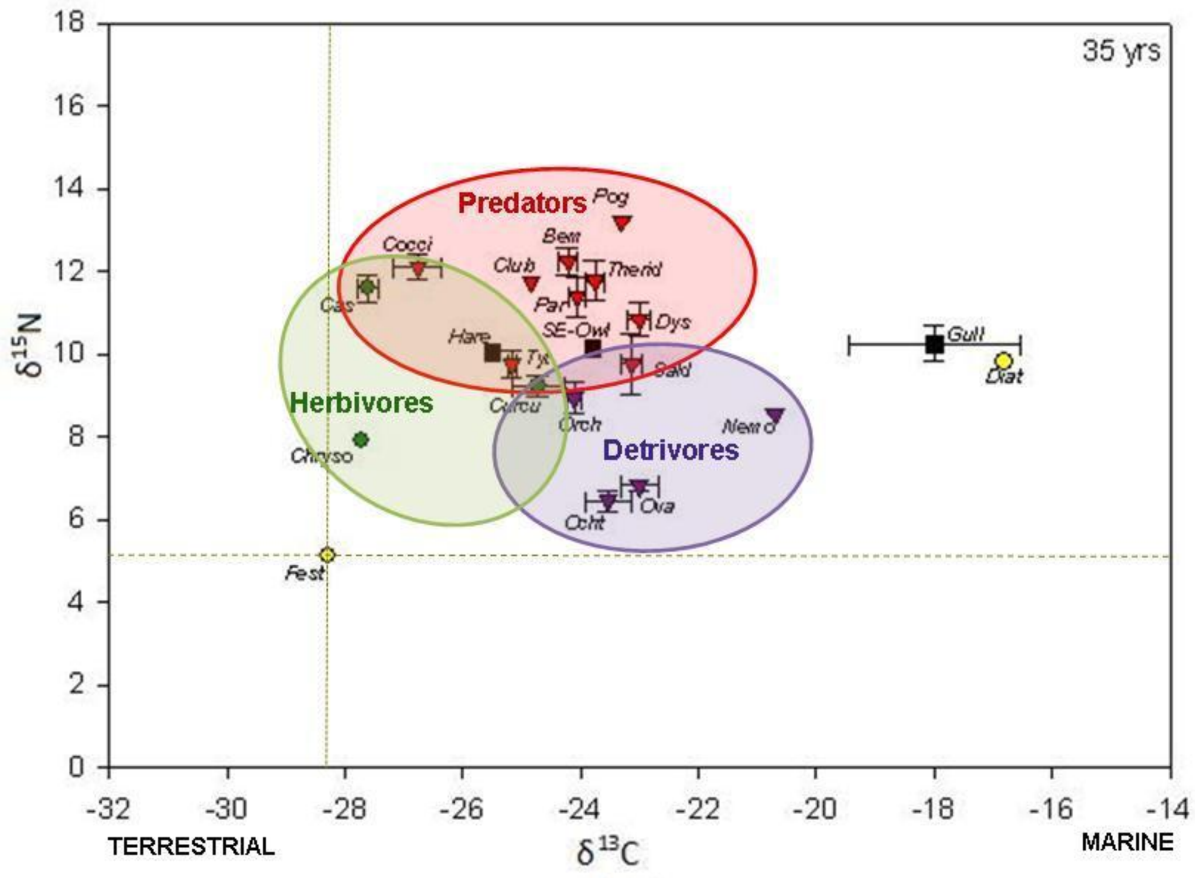


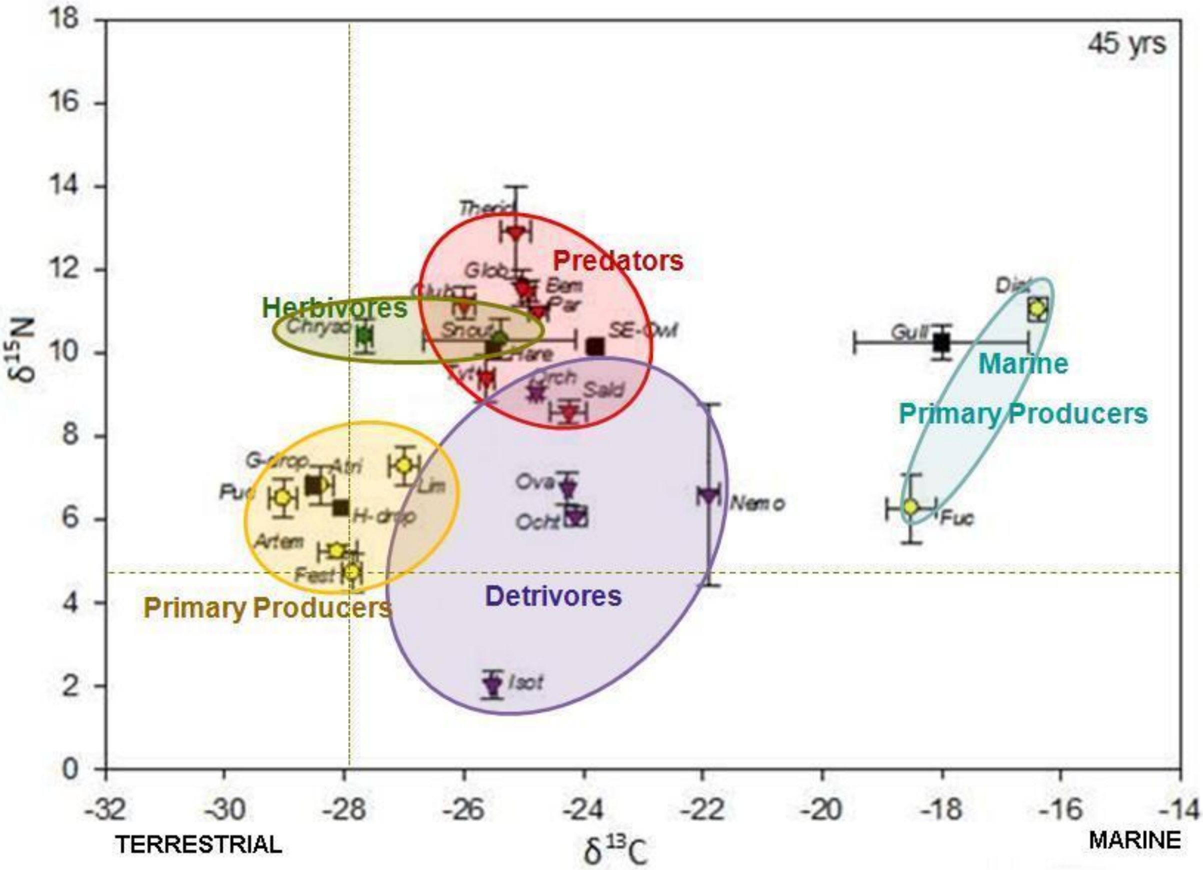
0 yrs
Enchyt
Sald
Hym
Therid
Scat
Dys
Predators
Club
Cas
Pog
Par
Hare
Herbivores
SE-Owl
Gull
Cocci
Diat
Tyt
Detrivores
Ble
Salic
Elat
Orch
Fest
TERRESTRIAL
MARINE
$\delta^{15}N$
$\delta^{13}C$

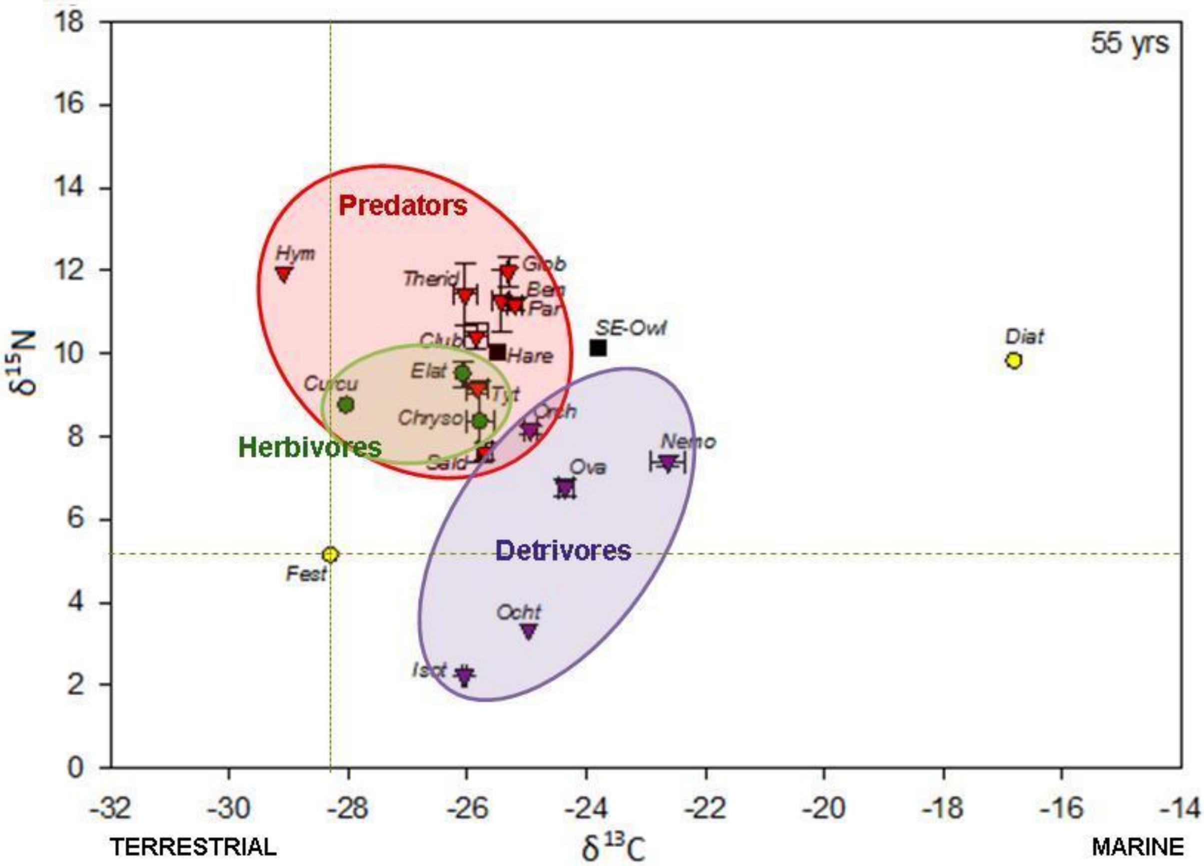


10 yrs
Predators
Therid
Club
Sald
Cas
Ben
Dys
Hare
Gull
Diat
Spar
Mug
Herbivores
SE-Owl
Primary Producers (C4)
Curcu
Pom
Plantago
Orch
Tyt
Nemo
Detrivores
Marine Primary Producers
Chryso
Ocht
Isot
Lim
Atri
G-drop
Ova
Fest
H-drop
Puc
Fuc
Primary Producers (C3)
TERRESTRIAL
MARINE
$\delta^{15}N$
$\delta^{13}C$


25 yrs
Predators
Herbivores
Detrivores
δ15N
δ13C
TERRESTRIAL
MARINE
Pog
Therid
Par
Club
Cas
Hym
Cocci
Hare
Bem
SE-Owl
Dys
Sald
Sym ol
Gull
Diat
Nemo
Orch
Wheatear
Acari
Chryso
Curcu
Ocht
Ova
Fest
Mecinus
Elat


35 yrs
Predators
Herbivores
Detrivores
δ15N
δ13C
TERRESTRIAL
MARINE
Pog
Cocci
Bem
Club
Therid
Cas
Par
SE-Owl
Dys
Hare
Ty
Sald
Gull
Diat
Curcu
Orch
Nemo
Chryso
Ova
Ocht
Fest


45 yrs
Predators
Herbivores
Marine
Primary Producers
Primary Producers
Detrivores
Therid
Glob
Bem
Par
Club
Chryso
Snout
SE-Owl
Hare
Tyt
Orch
Sald
Gull
Diat
Fuc
G-drop
Atri
Lim
Puc
H-drop
Artem
Fest
Ova
Ocht
Nemo
Isot
TERRESTRIAL
MARINE
δ¹³C
δ¹⁵N


55 yrs
Predators
Herbivores
Detrivores
Hym
Therid
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Bem
Par
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SE-Owl
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TERRESTRIAL
MARINE
δ¹³C
δ¹⁵N

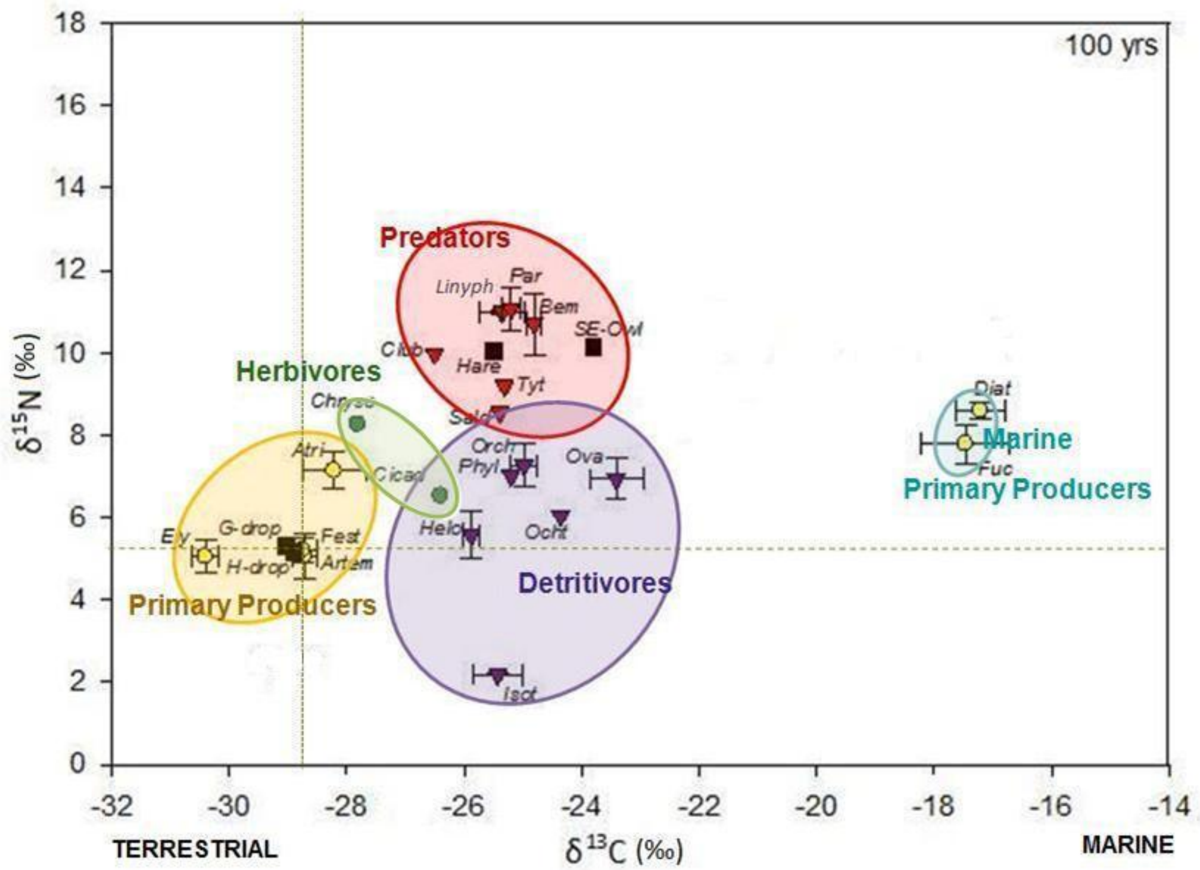

Figure 9a-9g: Trophic level ($\delta^{15}N$ ‰) and the degree of source input from the intern terrestrial salt marsh ecosystem and the extern marine Wadden sea ecosystem ($\delta^{13}C$ ‰) shown per succession stage (0 t/m 100 yrs). See for an overview of all species abbreviations in Appendix I.

Food web assembly: changes in $\delta^{13}C$ and $\delta^{15}N$ along succession

Internal fuelling: from open towards a closed system ($\delta^{13}C$ along succession)

When looking at the $\delta^{13}C$ position of detritivores along succession in Figure 9, the source signal ($\delta^{13}C$) of detritivores ranges between a terrestrial (low $\delta^{13}C$) and the marine (high $\delta^{13}C$) signal. Moreover, the detritivores do shift from a marine signal towards a terrestrial signal, this is visualized in Figure 12d and Figure 10 and can be statistically supported (Table 4: Change in stable isotope value per category along succession.

Not only detritivorous-, but also carnivorous invertebrates do change their food source from an external marine towards internal terrestrial fuelling system (especially see Figure 10 and Table 4, but also Figure 9 and Figure 12).

Also terrestrial primary producers do - according to statistical analyses - become more terrestrial orientated along succession (see Figure 10, Table 4 and Appendix III), but see discussion.

As expected, marine primary producers and herbivorous invertebrates do not change their $\delta^{13}C$ signal along succession.

SOM and TOM possibly follow a Gaussian distributed trend where the organic matter first becomes more terrestrial after which it would switch to a more marine signal again (see Appendix III).

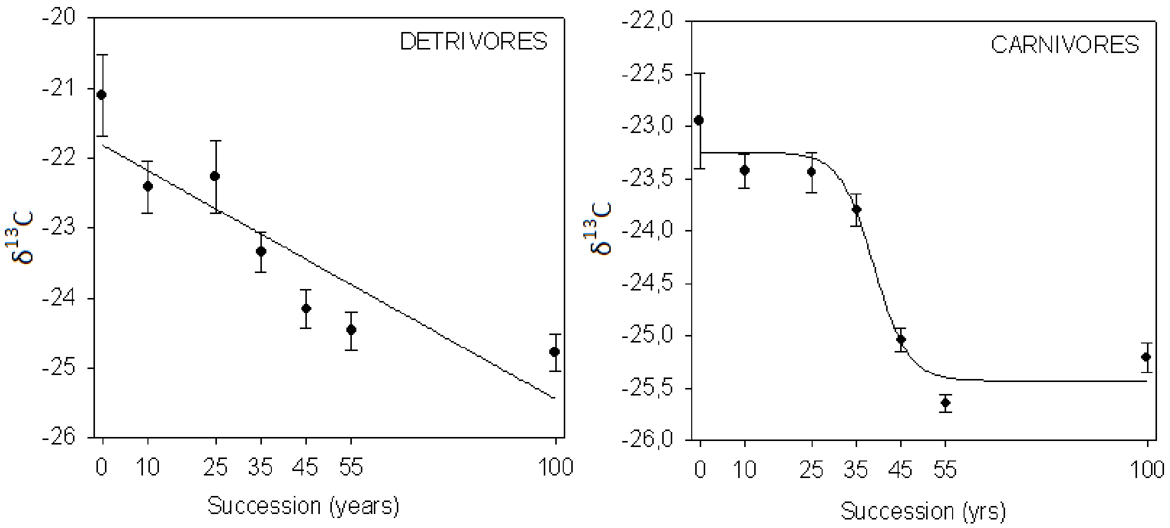

Figure 10: Detrivoreous and carnivorous invertebrates change their source along succession, from a marine- towards a terrestrial source (see Table 4 for statistical significance).

Changes in $\delta^{15}N$ and $\delta^{13C}C$ along succession General Linear Model (ANOVA) with covariate[species]					
	Sample[B]	$\delta^{13C}C$		$\delta^{15}N$	
	n	F	p	F	P
SOM[A]	3			0,01	0,946
TOM[A]	3			2,69	0,349
Ter primary producers[A]	15	6,78	**0,04**	0,44	0,532
Mar primary producers(All)[A]	8	0,19	0,686	0,41	0,557
Herbivorous invertebrates	22	0,00	0,965	0,85	0,373
Carnivorous invertebrates (All)	46	33,23	**<0,001**	9,86	**0,003**
Detritivorous invertebrates (All)	35	14,04	**<0,001**	4,81	**0,039**
A = measured along 3 succession stages(10, 45 and 100 years) instead of all 7 stages B = corrected for repeated replicates (replicate = if same species and plot).					

Table 4: Change in stable isotope value per category along succession

A decrease instead of an increase in trophic level ($\delta^{15}N$ along succession)

Although the hypothesis that only carnivorous and detritivorous invertebrates change in trophic level ($\delta^{15}N$) can be accepted (Table 4), the direction of this change was opposite to the expectations (Figure 11)!

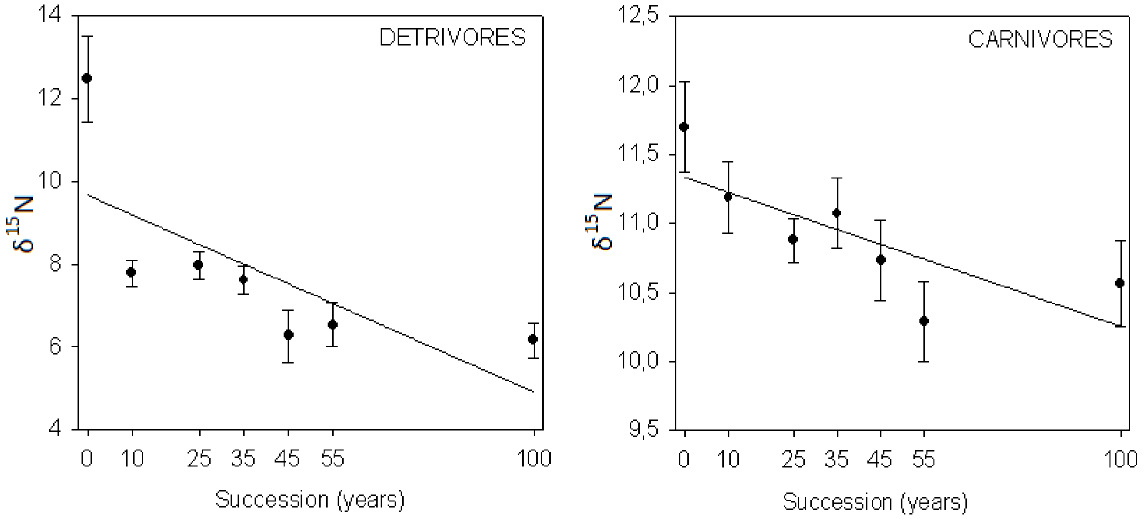

Figure 11: Detrivoreous and carnivorous invertebrates show a decrease in $\delta^{15}N$ along succession (see Table 4 for statistical significance).

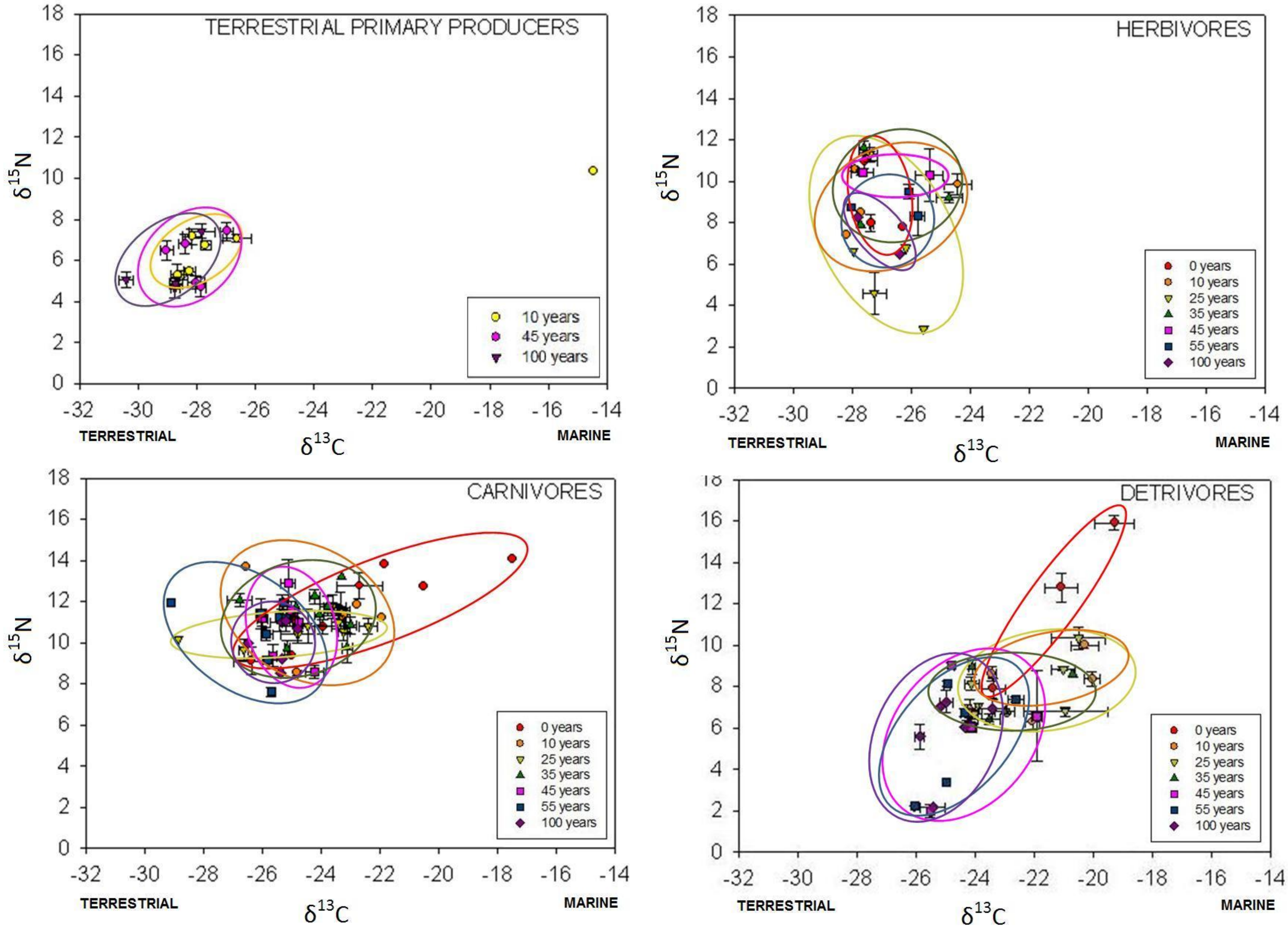

Figure 12a-12d: Change in $\delta^{13}C$ and $\delta^{15}N$ per category along succession

DISCUSSION

Simultaneously bottom-up & top-down regulation

Studies on vegetation succession on the salt marsh of Schiermonnikoog supported the bottom-up theory of Clements (1916) in which primary producers play the key role for ecosystem succession (Olff, De Leeuw et al. 1997; van Wijnen and Bakker 1997).

As Hairston (1960) emphasizes, energy in an ecosystem is build up by fixating carbon by primary producers. Internal energy and biomass of primary producers of an ecosystem therefore increases along succession. The bottom-up theory does therefore play a role in food web assembly along succession. However, this is not the only regulating mechanism of food web assembly. From 1997 onwards, it was already known that small herbivores (in this case hares) delay succession and change species composition on the salt marsh of Schiermonnikoog (Olff, De Leeuw et al. 1997; van Wijnen, van der Wal et al. 1999; van der Wal, van Wijnen et al. 2000). This would mean that not only bottom-up regulation, but also top-down regulation does influence food web assembly along succession.

In this study we showed that top-down regulation and external regulating factors also play an important role in food web assembly.

BOX IV : Sources do not differ in δ^{15}N and δ^{13}C along succession
Soil (SOM), terrestrial organic matter (TOM=dead plants) and marine organic matter (POM) had, as expected, a constant stable isotope signal along succession. Which means that each category has one trophic level and one source (marine or terrestrial). SOM and TOM showed a Gaussian trend with a mainly marine signal which would be terrestrial in the intermediate stage of succession. This unlikely result is probably due to the insignificant sample size of SOM and TOM together which limited the analysis in such a way that no the reliable statistical analysis could be run. The stable isotope signals of terrestrial primary producers were also expected to remain constant, which was true for the δ^{15}N since plants can only occupy one trophic level. However, terrestrial primary producers were found to change their nutrient source from the external marine, towards the internal terrestrial(change in δ^{13}C). This is, of course, high unlikely. Plants fixate most of their dry matter from the air (C), it seems therefore unlikely that terrestrial plants actually become more terrestrial. The actual reason of this decrease in δ^{13}C, might be that 1a) plant species composition along succession and δ^{13}C values differ between plant species and 1b) plant species with an r-strategy, which are characteristic for early successional stages, might structurally contain a higher δ^{13}C signal (so marine), compared with K-strategist which occurs in late succession. Furthermore, 2) plants in the early successional stages might –apart from the species - be more adapted to the marine system since they do live closer to the sea. The stable isotope signal herbivores (who can only feed on plants) did not change along succession. This supports the notion that plants do not actually change their C isotopic signal. In fact it does not matter if terrestrial plants change their carbon isotopic signal slightly, as the effect size of this change is very small, relative to the overall marine-terrestrial change. Except for the plant species *Spartina anglica* (see box VI), all plants gave a very terrestrial signal which contrasts strongly with the marine signal.

External factors influence food web assembly

Studies by Stapp & Polis (2003) and Page et al. (2008) already showed the importance of interaction of energy fluxes between ecosystems. In our study, we describes the importance of interaction between ecosystems for the understanding of the process of succession, in which external sources are used by the regarding ecosystem. In our case, the terrestrial salt marsh ecosystem is fuelled by the marine Wadden Sea ecosystem.

The general and most obvious idea of how marine input reaches the terrestrial salt marsh is by leaving marine organic matter behind after floodings of the Wadden Sea.

Polis (1995) described how an external ecosystems may supply the regarding ecosystem by not only water but also by guano (faeces) of extern birds. Our data strongly supports this hypothesis. Our field observations demonstrate that the abundance of marine birds are the highest in the pioneer stage. Marine birds do roost during high tide in the pioneer stage where they excrete guano. Since foraging marine birds were relatively rare (foraging was only observed for dunlins), netto, marine birds supply the pioneer salt marsh with more external Wadden Sea input than that they extract from the salt marsh ecosystem.

The reason why birds prefer the pioneer stage to other stages is unknown, but it might be a consequence of the slow tidal income of sea water which directs the birds ashore in the pioneer stage. Another plausible reason for roosting in the pioneer stage is the lack of vegetation, which supports vigilance for potential predators.

The underlying mechanism of this non-foraging behavior is also not known. Possibly, roosting birds are too busy with vigilant behavior or the pioneer stage lacks suiting food-items.

Potworms, *Enchytraea sp.* occur in the pioneer stage of succession and were found to have an extreme marine carbon signal and a high trophic level nitrogen signal. A plausible explanation for this would be that *Enchytraea* feeds on guano of marine birds. It thereby fixates external marine Wadden Sea source in the salt marsh ecosystem.

BOX V: Intermediate biomass peak

Especially herbivores - herbivorous invertebrates as well as other herbivores - do peak in the intermediate stage of succession. This seems to be contrary to 1) the asymptotic increase in plant biomass (Schrama, unpublished data, see Appendix VIII) and 2) the general assumption that total biomass will increase asymptotically along succession as overviewed by Odum (1969).

Whereas plant biomass increases along succession, the quality (N:C ratio) of the plant biomass decreases (see plant N:C ratios of the plant community change along succession on Mellem, a German Wadden island (see Appendix VIII)). If plant biomass increase and plant quality decrease along succession, the forage optimum for herbivores lays in the intermediate succession stage. This means that the bottom-up control, again, is important for food web assembly.

BOX VI: Why *Spartina* has a high $\delta^{15}N$ and $\delta\ ^{13}C$ ratios

Although only one *Spartina anglica* sample (from at least 5 different individuals) was analyzed, the $\delta^{15}N$ and $\delta\ ^{13}C$ ratios of this endophytic C4-plant *Spartina anglica* were extremely high ($\delta\ ^{13}C$:= -14,45 and $\delta^{15}N$ = 10,36) compared to other plants.

The reason for the higher $\delta^{13}C$ ratio is that during photosynthetic uptake of CO_2 , C4-plants fix more ^{13}C compared to C3-plants (Rao, Ayarza et al. 1994).

The high $\delta^{15}N$ ratio is probably due to the fact that *Spartina anglica* is an endophyte and thereby has the capacity to fix atmospheric nitrogen (2‰ to 8‰ $\delta^{15}N$) in its roots (Wigand, McKinney et al. 2007; Bai, Boutton et al. 2009). Furthermore our samples were taken in early spring. Previous studies found a higher $\delta^{15}N$ in spring compared to summer. They suggest that 1) spring rains and accelerating temperature rises in spring might trigger the N-cycle and thereby increasing the leaf $\delta^{15}N$ and 2) plants might first use their N-storage (spring) before using the nitrogen in the soil (generally lower $\delta^{15}N$ than atmosphere) in summer (See Filella & Penuelas 2003 and Chang & Handley 2000 in: Wigand, McKinney et al. 2007; Bai, Boutton et al. 2009).

Finally, the extremely high $\delta^{15}N$ ratio might also be due to enriched organic nitrogen in the soil and water, derived from livestock and human wastewater (10‰ to 22‰ $\delta^{15}N$) (Wigand, McKinney et al. 2007).

Another explanation would be that potworms exert a high tendency to retain ^{15}N, more so than other organisms. This hypothesis may be true since *Enchytraea* potworms were reported to relatively high in $\delta^{15}N$ in other ecosystems too (Scheu and Falca 2000; Schmidt, Curry et al. 2004).

So, whereas the terrestrial primary production is still minimal in the pioneer stage, external input can be fixated by detritivores.

In the pioneer stage of the salt marsh, terrestrial detritivores and carnivores both have quite a marine signal. This means that terrestrial carnivores feed on marine organisms (quite unlikely) or on terrestrial detritivores who fed on marine sources.

Our study goes further and proves that the salt marsh ecosystem closes its nutrient cycle along succession and becomes hereby a self-sustaining ecosystem. The isotope signal of detritivorous and carnivorous invertebrates changes along succession from a marine towards terrestrial signal. This means that whereas the pioneer stage still has a system with an open nutrient cycle, the ecosystem gradually closes its nutrient cycles along succession. The salt marsh ecosystem thereby becomes mostly independent of the interaction with the Wadden Sea ecosystem.

I think that most - and probably all - ecosystems do interact with adjacent ecosystems. I cannot think up an ecosystem where the pioneer ecosystem is not adjacent to another ecosystem. Since nutrient and energy availability is low in the pioneer stage, a logical step would be to derive this externally. As Odum (1969) already mentioned in his overview of the concept of succession, this interaction will weaken along succession because the regarding ecosystem has build up a proper internal nutrient cycle and energy cycle.

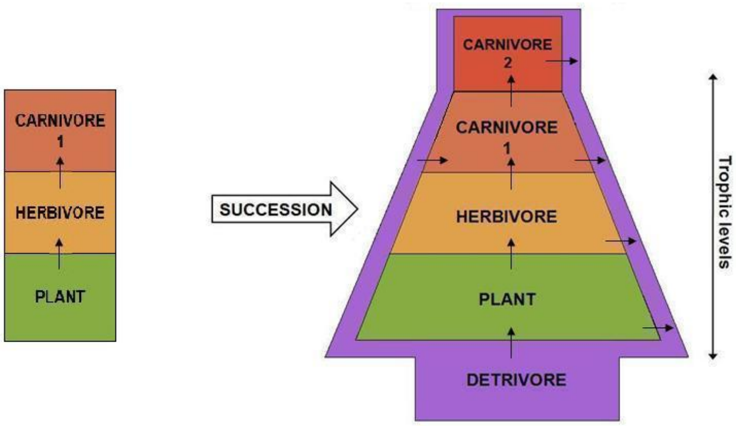

Figure 13a: Change in trophic structure along succession: Classical approach

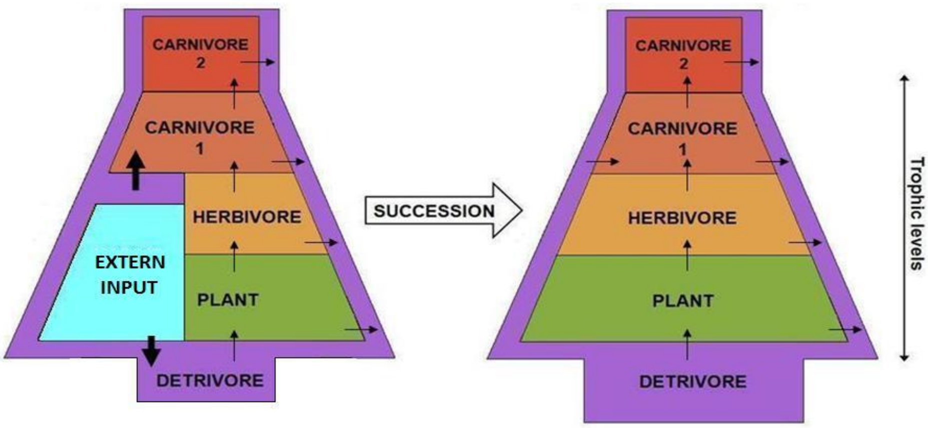

Figure 13b: Change in trophic structure along succession: Modern approach

Lacking an increase in trophic levels

Our empirical study did find no indication for a gain in number of trophic levels along succession as assumed in the theory of succession (Margalef 1967; Odum 1969). Our stable nitrogen isotope analyses even suggested a decrease in number of trophic levels. Field observations do not indicate a decrease in number of trophic levels. The probable reason for the decrease in $\delta^{15}N$ is the switch from a marine towards a terrestrial source (see for instance Figure 9 Figure 9 and Table 4).

A gain in number of trophic levels, can be achieved by an increase in secondary consuming carnivores or by addition of detritivores (which may feed on carnivores and function as prey for carnivores too). Detritivores are already present in the pioneer stage of the salt marsh where they feed on the external marine Wadden Sea which floods over the salt marsh every now and then or by guano of marine birds. Carnivores which are already present in a very early stage of succession, may feed on these marine feeding detritivores. This same carnivore species may switch diet along succession. This causes the decrease in $\delta^{15}N$.

In summary, the number of trophic levels remains constant, but the source input may change from a marine towards a terrestrial fed ecosystem (see Figure 13).

Top-down regulation influences food web assembly: Trophic- and species interactions

Although the number of trophic levels does not increase, the (importance of)interactions between trophic levels probably does change along succession. We did find a number of clear examples of (top-down) interactions between trophic levels and between species of the same trophic level, which I will now discuss. All these interactions are in some way or another of influence on food web assembly.

Invertebrate-feeding birds:

Abundance of the invertebrate-feeding birds and small mammals (voles, but also hare and geese - see Appendix VIII) all peak in intermediate succession stages.

This peak of invertebrate-feeding birds can be explained by the invertebrate study of Schrama, who described a peak in the intermediate succession stage for the biomass of the aboveground invertebrate community (Schrama, unpublished data, see Appendix VIII). For an explanation of the invertebrate peak in the intermediate stage of succession, see box V.

Birds and mammals (higher trophic organisms) feed on lower trophic organisms and thereby control the food web assembly of - in this case - the intermediate stage of succession.

BOX VII: Solving enigmatic isotope values
Isotoma riparia springtails - which occur in stage 45, 55 and 100 yrs - do have an extremely low $\delta^{15}N$ value, which may be due to a microbial diet. Coyle et al. (2009) describe that $\delta^{15}N$ values of the microbial community drops with increasing C:N ratios. Biomass quality (expressed in C:N ratio) decreases on the salt marsh along succession. This supports our hypothesis that *Isotoma riparia* have a microbial diet.

***Gull-Jackdaw*-ecosystem**: A more explicit influence of birds on its community occurs in the 35 years old succession stage. Here, the abundance of invertebrate-feeding bird is abnormally low. The reason of this low abundance is a big gull colony near the 35 year old stage. Gulls are very territorial and do tolerate practically no other birds. Jackdaws, though, - which are rather abundant in stage 35 - are known to be tolerated by gulls and they do breed in gull colonies. So, higher trophic organisms compete, whereby the best competitor (gulls) controls the food web assembly (in this case invertebrate-feeding birds, which subsequently also have an influence on the underlying food web).

Raptors: In the classical approach of food web assembly along succession (Odum 1969; Oksanen 1981), raptors would become abundant in late succession. Our study, though, shown that raptors occur along the whole successional gradient. There are a couple of possible explanation for this finding. Firstly, raptors do have a big home range and may therefore be seen in pioneer succession stages, while feeding in later successional stages or in higher elevated gradients of the salt marsh. Secondly, raptors may actually feed on prey from the pioneer stage. The presence of this prey can be explained by external input, as described earlier before. Thirdly, the prey might be present in the pioneer stage, but forage somewhere else.

The observations of this study were not suiting for estimating raptor abundance. A half of an hour observations is way too short to gain insight in the occurrence and feeding spots of raptors. Since raptors have a quite a large home range, they may only fly over without hunting.
On the other hand, no matter what the reason of the raptors abundance might be and no matter whether or not raptors actually hunted when flying by, they do disturb the bird fauna (personal observations).

Feral cats: Although it is not sure whether or not the feral cats did kill the (weakened) hare, our results indicate that feral cats do certainly feed on small mammals and birds.

Further research is required to investigate the impact and abundance of feral cats on salt marsh fauna. We propose to quantify the abundance of feral cats by using infrared-cameras and cat mint oil as an attractant. For the diet investigation, cat droppings need to be determined more precisely and investigation in the 'by feral cats eaten carcasses'-database should be extended.

Detrivore-vole interaction: Another influence - of a totally different caliber - on food web assembly, are the detritivores *Ovatella myosotis* and *Orchestia gammarella* – which were high abundant in the mice holes in the intermediate stage of succession. These detritivores high probably feed on nutrient-concentrated vole droppings. Voles hereby possibly facilitate detritivores. This symbiosis is a form of positive top-down regulation. In general, symbiosis is thought to increase along succession (Odum 1969). It would therefore be curious to research this possible vole-detritivores symbiosis more into detail and to research its influence on succession.

Detrivore-regulation plays a key role in food web assembly

Odum (1969) describes that especially the climax stage is regulated by detritivores. We certainly do not contradict this, but we are happy to add the importance of detritivores in early succession stages. As visualized in Figure 13, our results indicate that detritivores exert an even more important role in the pioneer stage than in the climax stage of succession. That is, they play a role in fixing the external marine source from the marine organic matter or from guano, which no doubt speeds up the production of terrestrial vegetation.

But as described above, numerous detritivores-included interactions were found to play a regulating role in the food web assembly in later succession stages too.

CONCLUSIONS BASED ON OUR EMPIRICAL STUDY	
How does a food web assemble along succession?	
Food web complexity	~~Development from a simple food chain (plant-herbivore-carnivore) towards a complex and detritivorous-pathway-controlled food web.~~
	It is yet unclear whether or not food web complexity increase along succession, since the pioneer stage is already rather complex due to interactions with extern ecosystems. Moreover, all over the succession gradient, many interactions between organisms occur.
Trophic structure	The number of trophic levels ~~will~~ do not increase along succession.
	All trophic levels are already present in the pioneer stage. See Figure 13.
Food web regulation	Food web assembly is regulated by bottom-up control & top-down control.
Does the salt marsh ecosystem changes from an open towards a closed regulated ecosystem?	
Nutrient cycle	The open nutrient cycle in the pioneer stage will gradually close its cycle along succession, which means that the ecosystem develops to a self-sustaining climax stages which needs no external source input anymore. >> The interaction between the terrestrial salt marsh and the marine Wadden sea ecosystem will weaken along succession.

Table 5: Answers on the research questions, as given in the introduction

Since the biomass of microbivores and macro-detritivores both increase linear along salt marsh succession (see Appendix VIII), it would be surprising if more interactions would occur along succession.

Conclusion

Considering abovementioned interaction of higher trophic organisms, our study shows that higher trophic levels do regulate food web assembly.

The results of the abundance of all species in combination with stable isotope analyses on the salt marsh, as researched in this study, allows us to make the first step towards a theory of food web assembly along succession, including both a bottom-up and a top-down approach. In this modern theory of food web assembly along succession, higher trophic levels, detritivores and external ecosystems play an important regulating role in ecosystem assembly.

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