Icelandic grasslands as long-term C sinks under elevated organic N inputs

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Abstract. About 10 % of the anthropogenic CO_2 emissions have been absorbed by northern terrestrial ecosystems during the past decades. It has been hypothesized that part of this increasing carbon (C) sink is caused by the alleviation of nitrogen (N) limitation by increasing anthropogenic N inputs. However, little is known about this N-dependent C sink. Here, we studied the effect of chronic seabird-derived N inputs (47–67 kg N ha^{-1} yr^{-1}) on the net soil organic C (SOC) storage rate of unmanaged Icelandic grasslands on the volcanic Vestmannaeyjar archipelago by using a stock change approach in combination with soil dating. We studied both early developmental (young) soils that had been receiving increased N inputs over a decadal timescale since an eruption in 1963, and well-developed soils, that had been receiving N inputs over a millennial timescale. For the latter, however, the effects on both decadal (topsoil; 40 years) and millennial (total soil profile; 1,600 years) SOC storage could be studied, as the age of topsoil and the total soil profile could be determined from volcanic ash layers deposited in 1973 and 395 AD. We found that enhanced N availability - either from accumulation over time, or seabird derived - increased the net SOC storage rate. Under low N inputs, early developmental soils were weak decadal C sinks (0.018 ton SOC ha^{-1} yr^{-1}), but this increased quickly under ca. 30 years of elevated N inputs to 0.29 ton SOC ha^{-1} yr^{-1}, thereby equalling the decadal SOC storage rate of the unfertilized well-developed soils. Furthermore, for the well-developed soils, chronically elevated N inputs not only stimulated the decadal SOC storage rate in the topsoil, but also the total millennial SOC storage was consistently higher. Hence, our study suggests that Icelandic grasslands, if not disturbed, can remain C sinks for many centuries under current climatic conditions and that chronically elevated N inputs can induce a permanent strengthening of this sink.

Keywords: Terrestrial C sink, N inputs, Long-term carbon storage, Soil development, Surtsey

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1 Introduction

The global C cycle plays a prominent role in climate change and is greatly influenced by anthropogenic C emissions (IPCC 2013). During the past 20 years, terrestrial ecosystems have been absorbing ca. 30 % of the total anthropogenic C emissions; a sink that has been increasing (Le Quere et al. 2009; IPCC 2013). However, the future evolution of the terrestrial sink-source balance is highly uncertain, and depends on a multitude of factors, such as land use and nutrient dynamics (Poulter et al. 2011; IPCC 2013; Fernandez-Martinez et al. 2014; Wieder et al. 2015).

Northern mid- and high latitude terrestrial regions (>50° N) are important C sinks (Ciais et al. 1995; Poulter et al. 2011), accounting for about 30 % of the global net terrestrial C uptake (White et al. 2000). It has been hypothesized that this observed C sink is to a large extent caused by the alleviation of widespread N limitation in these ecosystems (Hudson et al. 1994; Lloyd 1999; Schlesinger 2009) due to the three to five fold increase in anthropogenic N deposition during the past century (Galloway et al. 2008; Gundale et al. 2014). Although a further increase in high-N deposition is projected at northern latitudes (IPCC 2013), the continuation of the northern C sink is highly debated, with estimates ranging from a current decline to a steady increase until at least the middle of this century (Lloyd 1999; White et al. 2000; Cramer et al. 2001; Bachelet et al. 2003; Pepper et al. 2005; Friedlingstein et al. 2006; Canadell et al. 2007; Morales et al. 2007; Le Quere et al. 2009; Tao & Zhang 2010; IPCC 2013; Todd-Brown et al. 2013; Arora & Boer 2014). This underlines the necessity for a better understanding of the N-induced stimulation of long-term C storage in northern ecosystems.

Uncertainties in the further development of this N-dependent northern C sink are mainly related to the limited number of long-term N fertilization studies. The rare long-term studies that have studied ecosystem SOC stocks report contradicting conclusions, which vary with fertilization rate and litter quality (Hyvonen et al. 2007; Hopkins et al. 2009; Nilsson et al. 2012; Gundersen et al. 2014). In contrast, a large number of short-term N addition studies have been performed at high latitudes and many of those have investigated aboveground C input fluxes (GPP) and ecosystem C output fluxes (litter decomposition and heterotrophic and autotrophic respiration; Wookey et al. 2009; Bouskill et al. 2014). However, such short-term studies are not able to include potential N saturation effects and slower acting mechanisms and can therefore not fill the knowledge gap on chronical N input effects on ecosystem C dynamics.

A second factor that greatly increases the uncertainty about the further evolution of N-dependent northern C sink is the fact that the developmental stage of ecosystems is often neglected in studies on net ecosystem C storage

(Kroël-Dulay et al. 2015). Ignoring developmental stage likely contributes to a substantial part of the uncertainties in global C dynamics (Chapin et al. 2011). For instance, the link between elevated N inputs and net ecosystem C storage might change during the course of soil development (Crocker & Major 1955; Saynes et al. 2005; Seedre et al. 2011; Appling et al. 2014). This is especially true for N-limited ecosystems (Aerts & Chapin 2000; Reich & Oleksyn 2004), where total N stocks and plant available N increase during the process of soil development (Crocker & Major 1955; White et al. 2004; Rhoades et al. 2008; Smithwick et al. 2009).

Unmanaged northern grasslands have an extensive coverage (10 % of the global terrestrial surface; Chapin et al. 2011) and have a large SOC storage potential (Aerts et al. 2003; Sui & Zhou 2013), whereof >95 % of the total C is stored as SOC (Grace 2004). Moreover, it is likely that the response of these typically N limited systems (LeBauer & Treseder 2008) to long-term N fertilization will be more pronounced than that of more southern grassland sites with higher background N deposition (Hopkins et al. 2009). However, the role of chronic N inputs in the net SOC storage in these ecosystems is yet unclear.

In this study we therefore investigated the effect of chronically elevated N inputs on the C sink of northern soils of contrasting developmental stage, by quantifying the total ecosystem C stocks and net SOC storage rates of unmanaged Icelandic grasslands. We studied two site pairs with contrasting chronical N inputs (of decadal (medium-term) and millennial (long-term) timescale), one pair with soils of an early developmental stageand one pair with well-developed soils. All sites were located on the volcanic Vestmannaeyjar archipelago (south of Iceland, Fig. 1). Variations in natural N inputs were caused by the preferences of seabirds to form breeding colonies (which are hotspots of seabird-derived N inputs; Garcia et al. 2002; Zwolicki et al. 2013; Havik et al. 2014) at specific locations. Different soil developmental stages were included by studying a young island (Surstey, 50 years old) and two older islands (Heimaey and Ellidaey, both with an undisturbed soil profile of 1,600 years). The duration of the increased N inputs on a part of the young island was 26 years (decadal), while this was likely close to 1,600 years (millennial) for the older island Ellidaey.

Firstly, we expected that increased seabird-derived N inputs would increase soil N stocks and N availability, both in early developmental soils and well-developed soils. In addition, we expected that the effect of increased N inputs on net SOC storage (see next hypothesis) would overrule the effects of any other factor that is known to influence net SOC storage because of the strong N limitation of these Icelandic grasslands (Leblans et al. 2014; Magnússon et al. 2014).

We hypothesized that decadal N inputs would stimulate the net SOC storage rate of both the early developmental and the well-developed Icelandic grassland soils, in accordance with what was observed at mid- and high latitudes during the past decades (Hudson et al. 1994; Lloyd 1999; Schlesinger 2009). Further, this stimulation was expected to be more pronounced for early developmental soils, because of their relatively higher N limitation. Millennial N inputs were hypothesized to persistently stimulate the net SOC storage, but to a smaller extent than the decadal N inputs as a consequence of SOC saturation processes in the subsoil (Zehetner 2010; Olson & Al-Kaisi 2015). These hypotheses result in the following orders of net SOC storage rate on decadal timescale: early developmental soils with low N inputs <<<< early developmental soils with high N inputs; well-developed soils with low N inputs << well-developed soils with high N inputs; and on millennial timescale: well-developed soils with low N inputs < well-developed soils with high N inputs.

2 Material and methods

2.1 Study sites

This study was performed on three islands of the volcanic Vestmannaeyjar archipelago (63°25’ N, 20°17’ W; south Iceland; Fig. 1) in mid-July 2012 and 2013. The climate is cold temperate, with a mean annual temperature at Stórhöfði (meteorological station on the main island, Heimaey) of 5.1 °C between 1963 and 2012, and a min. and max. monthly average of 1.3 and 9.6 °C. Mean annual precipitation during the same period was 1600 mm (Icelandic Meteorological Office; www.vedur.is). The main vegetation type on the Vestmannaeyjar archipelago are lush grasslands, except in areas that are unsuitable for seabird colonization where heathlands, herb slopes or dry meadows can be found (Magnússon et al. 2014).

Two pairs of sites with low and high natural N inputs were established on islands where soils were either at an early developmental stage (E) or on islands with well-developed soils (W) (Magnússon et al. 2014). Variations in natural N inputs were caused by the preferences of seabirds to form breeding colonies (which are hotspots of seabird-derived N inputs; Garcia et al. 2002; Zwolicki et al. 2013; Havik et al. 2014) at specific locations. As N is by far the most limiting element in the ecosystems under investigation (Aerts & Chapin 2000; Leblans et al. 2014), the influence of other seabird derived nutrient inputs was assumed to be negligible. Also the organic C content of seabird guano (~ 25% at our study sites; R. Aerts, personal communication) should be taken into consideration when studying C storage. Nonetheless, the influence of seabird-derived organic C inputs on total C storage was assumed negligible, as it was found to account for only 1.2 to 1.8% of the total organic C inputs (assuming a vegetation turnover rate of 1 year, a realistic value for alpine grasslands; Perez & Frangi 2000). Further, the size of this contribution is not expected to change during the C stabilization process, as previous studies found no difference between the C storage efficiency of biomass residues and bird manure (Rahman 2013; Hua et al. 2014; Rahman 2014).

The low N sites (E_{NL} and W_{NL}) received on average 1.3–1.4 kg N ha^{-1} yr^{-1} in the form of natural background N input by atmospheric deposition (Sigurdsson & Magnússon 2010). No symbiotic N_2 fixing vascular plant species were found in any of the study plots (Magnússon et al. 2014). The high N input sites (E_{NH} and W_{NH}) received on average 47 kg N ha^{-1} yr^{-1} in the case of E_{NH} (Leblans et al. 2014) and 67 kg N ha^{-1} yr^{-1} in the case of W_{NH}, an estimation based on a bioenergetics model of Wilson et al. (2004) and Blackall et al. (2007) in combination with nesting densities from Hansen et al. (2011). All sites had similar bedrock characteristics (see further) and were located within 25 km distance from each other so that the influence of climate could be assumed to be negligible.

Variation in the developmental stage of the soils and in the duration of the N inputs (decadal vs. millennial) was included by selecting islands of different age. The E_{NL} and E_{NH} sites were located on the island Surtsey (Fig. 1), a 50-year-old volcanic island that was formed during an eruption between 1963 and 1967. While E_{NL} was virtually free of seabird influence, E_{NH} was located inside the confines of a well-defined permanent breeding colony of lesser black backed seagulls (*Larus fuscus*), great black-backed gulls *(Larus matitiumus*) and herring gulls (*Larus argentatus*) that was established in 1986 on the southwestern part of the island (Leblans et al. 2014; Magnússon et al. 2014). Limited soil formation had taken place at E_{NL}, while the E_{NH} soil profile consisted of an O horizon, on top of a premature A horizon (max. 10 cm deep) and was classified as an Andosol (Arnalds 2015). The pH at E_{NL} was significantly higher than at E_{NH} (7.6 vs. 6.6; Sigurdsson & Magnússon 2010). The plant community at E_{NL} was in an early successional transitional state between barrens and grassland, while the plant community at E_{NH} had reached an early successional grassland stage (Magnússon et al. 2014). Both E_{NL} and E_{NH} were located on the lower plain of basaltic lava flows that are partly filled with sand and silt (Jakobsson et al. 2007).

The W_{NL} sites were located on Heimaey, the largest island of the Vestmannaeyjar archipelago (13.4 km^2) (Fig. 1). It was established in Lyngfellisdalur, a valley on the southeastern part of the island. The valley is visually isolated from the sea, which makes it an unsuitable breeding location for seabirds. No seabird colonies were found within the valley and it is highly unlikely that they ever existed in the past because of the topographical conditions. The surfacing basaltic bedrock dates back to 5,900 BP (Mattsson & Hoskuldsson 2005) and is covered by a well-developed soil classified as ‘Brown Andosol’ (Arnalds 2008), which typically have a pH between 5.5 and 7.5 (Arnalds 2015). The W_{NL} sites host species-rich grassland communities, typical for low nutrient conditions (Magnússon et al. 2014). The W_{NH} sites were located on the nearby island Ellidaey (0.46 km^2) (Fig. 1) which hosts the second largest puffin colony (*Fratercula arctica*) of the archipelago, with 16,400 breeding pairs (Hansen et al. 2011). Due to its topographical conditions it is highly likely that the island has served as breeding ground for seabirds from early times. The W_{NH} sites had similarly aged bedrock and soil characteristics as the nearby W_{NL} sites on Heimaey (Mattsson & Höskuldsson 2003; Magnússon et al. 2014), but the nutrient-rich conditions have given rise to the development of a species-poor grassland community (Magnússon et al. 2014).

The soils of W_{NL} and W_{NH} contained two well-defined volcanic ash layers that could be used to date the profile; the lower one from a volcanic eruption in ca. 395 AD, which most probably originated from the mainland volcano Katla (Larsen 1984), while the upper one originated from an eruption on Heimaey in 1973 (Morgan

2000). Both ash layers varied in thickness between 0.5 and 5 cm. The 395 AD layer was located at 110 ± 5 (SE) cm soil depth at W_{NL} and at 160 ± 5 (SE) cm soil depth at W_{NH}, and coincided with the maximum depth of undisturbed soil, as below it an eroded gravel layer was found. The 1973 AD layer was located at 6.4 ± 0.4 (SE) and 11.4 ± 1.7 (SE) cm soil depth at W_{NL} and W_{NH}, respectively, and could be considered as the separation between topsoil and subsoil. At both sites, the topsoil contained over 70 % of the root biomass. At E_{NL} and E_{NH}, the vegetation and soil development was too recent to detect the 1973 AD ash layer, and, as no subsoil was developed yet, all soil was considered as topsoil.

2.2 Experimental setup

At E_{NL} and E_{NH}, all measurements were performed at ten and eight permanent 10x10 m survey plots, respectively. These permanent survey plots were established on Surtsey between 1990 and 1995 (Magnússon et al. 2014) and have been followed closely ever since, yielding an extensive amount of published data on important background variables such as vegetation development, seabird nesting history, soil parameters and gas exchange. To keep the permanent survey plots undisturbed for future research, destructive soil- and vegetation sampling (see § 2.4, 2.5 and 2.6) were always done outside the confines of the permanent survey plots. Therefore, three 0.2x0.5 m subplots were placed adjacent to each permanent survey plot. In both W_{NL} and W_{NH}, three 0.2x0.5 m subplots were placed adjacent to four 10x10 m research plots (n=4) that were established in 2013 and will be used in the future for further research. The subplots at W_{NL} and W_{NH} were protected against possible human and livestock influence prior to the measurements (early May – late July) by covering them with 1x1 m enclosure cages. Prior to the installation of the sheep-exclosures, the grazing pressure of W_{NL} and W_{NH}, was similar. Therefore, we assume that no major differences in trampling and consequential disruption of soil aggregation took place and that the grazing influence on C storage was minor. Further, additional C and N inputs from livestock were minimal, as no extra feeding was provided, and no signs of major C and N redistributions were observed, as grazing and manure dropping were not separated in space. No such protection against livestock was needed for E_{NL} and $E_{NH,}$ since neither tourists nor domestic animals are permitted on the Surtsey island (Baldurson & Ingadóttir 2007). Nevertheless, also the sites with early developmental soils could be considered 'grazed', as graylag geese have colonized the island, feeding upon the grasslands there (Magnússon et al. 2014).

2.3 N availability

A relative measure for N availability was obtained using cation- and anion-exchange membranes (PRS™ probes, Western Ag Innovations Inc.; Saskatoon, SK, Canada). The membranes continuously absorb charged ionic species over the burial period, and the N availability is calculated as soil N flux over time. Four sets of membranes were inserted for one week in the topsoil (0–10 cm depth) of each main study plot in mid-July 2013. Afterwards, they were sent to Western Ag Innovations Inc. (Saskatoon, SK, Canada) for further analyses.

2.4 Plant analyses

Vegetation cover (monocots, dicots and moss) was determined in the permanent (10x10 m) plots using a line-intercept method (Magnússon et al. 2014). Vegetation biomass was determined by harvesting all aboveground parts of vascular plants were in each 0.2x0.5 m subplot, and collecting litter and moss in a 0.2x0.2 m section of the subplot. Subsequently, all vegetation samples were dried for 48 h at 40°C or until weight loss stopped, weighed, and milled using a ball mill (Retsch MM301 Mixer Mill, Haan, Germany) in preparation for further C and N analyses by dry combustion (Macro Elemental Analyser, model vario MAX CN, Hanau, Germany).

In each of the permanent survey plots, the height of three individuals of *Cerastium fontanum* was measured, as it was the only plant species that was common between all four treatments. Further, 2 g dry weight of mature healthy leaves of *C. fontanum* were collected for analyses of N by dry combustion (NC2100 C/N analyser; Carlo Erba Instruments, Italy) and of P by inductively coupled plasma procedure (sequential ICP-OES spectrometer; Jobin Yvon Ultima 2, France), respectively.

2.5 Soil analyses

For all plots, soil depth was measured with a probe at 30 cm intervals along the sides of each plot and averaged. Underneath the subplots for vegetation sampling (see Sect. 2.4), two parallel soil cores (8.67 cm diameter) were taken and split into 0–5, 5–10, 10–20 and 20–30 cm depth segments, where depth to the bedrock allowed this at E_{NL} and E_{NH}. At W_{NL} and W_{NH}, two additional 4.82 cm diameter soil cores were extracted down to the 395 AD ash layer from three out of the four main plots with a closed split corer and separated in segments of 30 cm. Each pair of soil cores was used to retrieve the dry weight and C and N concentrations of the fine roots and of the soil fraction < 2 mm, to calculate the stoniness of the soil (the soil fraction > 2 mm; the C and N content of soil particles > 2 mm was assumed to be negligible) and the soil bulk density (BD) and to analyse the grain size distribution of the soil and the soil pH. From the first of the two soil cores, the fine roots were washed out on a 0.5 mm sieve and subsequently treated identically to the aboveground vegetation. From the same core, the stoniness of the soil (fraction of soil particles > 2 mm) was derived using a sieve with a mesh size of 2 mm. The second soil core was dried for 48h at 40°C or until weight loss stopped, and the soil bulk density (dry weight of soil < 2 mm per sampling core volume) was calculated using the following equation:

$$BD\ s_{<2mm} = \frac{(DW\ s_{total} - DW\ s_{>2mm} - DW\ r)}{V\ s_{total}} \tag{1}$$

Where $BDs_{<2mm}$ is the bulk density of the portion of soil particles < 2 mm, DW is dry weight, s_{total} is total soil core, $s_{>2mm}$ is the portion of soil particles > 2 mm, and r are the fine roots and V is the volume.

Afterwards 2 g of soil < 2 mm was sieved from the second soil core and milled with a ball mill (Retsch MM301 Mixer Mill, Haan, Germany) as preparation for further C and N analyses.

A second aliquot of ~ 2 g of dried soil < 2 mm was sieved from the second soil core for soil texture analyses. Prior to the analyses, organic matter was removed from the samples by treatment with H_2O_2 and iron oxides were disrupted by HCl in accordance to (Pansu 2003). Subsequently, all samples were analysed for grain size distribution using a particle size analyser (Mastersizer 2000, Malvern instruments, United Kingdom). Finally, a third aliquot of the same soil (but only for 0-10 cm soil depth) was used for pH_{H2O} determination. The samples were dissolved in a 1:2.5 (per mass) solution, shaken for 20 min and shaken shortly again after two hours before measuring pH with a Two Channel Benchtop pH/mV/ISE Meter (Hanna Instruments, Temse, Belgium).

2.6 Calculation of C and N stocks and net SOC storage rates

The C and N stocks (in ton ha^{-1} and kg ha^{-1} respectively) of vegetation and roots were calculated by multiplying the respective C and N concentration with the dry weight of the sample and correcting for the respective sampling size and depth. The C and N stocks of the soil were calculated by multiplying the respective C and N concentration with the bulk density of the soil (see equation 1) and transformed to the same units by correcting for the respective core size and depth.

The net SOC storage rate was calculated for both the topsoil (decadal net SOC storage rate) and the total soil profile (millennial net SOC storage rate). The topsoil was defined as the soil layer on top of the 1973 ash layer (accumulated over 40 years and containing > 70 % of the roots; see § 2.1) at the sites with well-developed soils (W_{NL} and W_{NH}). At the early developmental sites (E_{NL} and E_{NH}), no subsoil had been formed yet. Therefore the total soil profile, which had a comparable age as the topsoil of the well-developed soils (45 years, accumulated since the eruption ended in 1967), was classified as topsoil. For the sites with well-developed soils, the total soil profile reached until the 395 AD ash layer (see § 2.1).

The decadal (topsoil) net SOC storage rate (ton SOC ha^{-1} yr^{-1}) was calculated by dividing the topsoil SOC stocks by their respective accumulation time in year. This was 45 years for E_{NL}, 40 years for $W_{NL(D)}$ and $W_{NH(D)}$ (see earlier). For E_{NH}, where the soil had been accumulating under increase N inputs for ~26 years (since the initial seabird colonization of each plot (1986-2012; Magnússon et al. 2014)), the net SOC storage rate was calculated by firs correcting the SOC stock for the amount of SOC accumulated before the start of the colonization, which was derived from the stocks in the E_{NL} sites (assuming a constant accumulation rate) and dividing the resulting SOC stock by the respective accumulation time. The millennial (total soil profile) net SOC storage rate at $W_{NL(M)}$ and $W_{NH(M)}$ was calculated for consecutive cumulative soil ages, with 200 years intervals, down to the 395 AD ash layer. The age of the soil was calculated by assuming a constant soil accumulation rate between the two ash layers (0.67 and 0.97 mm yr^{-1} at W_{NL} and W_{NH}, respectively). The time resolution of 200 years was chosen because a detailed dating of soil profiles in S-Iceland (distant from active soil erosion areas (Gisladottir et al. 2010)), showed a reasonably stable average accumulation rate over this time scale. The reader should, however, be aware that this is an imprecise calculation.

2.8 Data analyses

First the overall effect of N input rate, soil depth, pH, vegetation (grass/herb ratio and moss cover) and soil texture (clay, silt and sand content) on the net SOC storage rate was tested with a multiple regression with net SOC storage as response variable. This model was sequentially simplified by excluding non-significant factors. Then the effects of the chronically elevated N inputs and soil developmental stage on N stocks, N availability, total aboveground biomass, N/P ratio and plant height of *C. fontanum* were tested with a two-way ANOVA, with N input (low/high) and soil developmental stage (early/well-developed) as fixed factors. In case of significant interaction, the pairwise differences were tested by post hoc LSD tests or Wilcoxon signed rank tests when the requirements of normality and homoscedasticity were not met. Finally, the interactions between the significant factors were included in the model and again excluded when not significant. The effects of decadal elevated N inputs and soil developmental stage on ecosystem C stocks and net SOC storage rate, were tested with a two-way ANOVA as described above. The change in net SOC storage rate under millennial elevated N inputs (for cumulative soil ages with 200 years interval, see § 2.6) was tested for W_{NL} and W_{NH} with a two-way ANOVA, with N input (low/high) and cumulative soil age as fixed variables. The correlation between net SOC and net SON storage rate was tested with a Pearson correlation test (conditions of normality and homoscedasticity were met). All tests were performed in R software (R-core-team 2014) and null hypotheses were rejected at $p < 0.05$.

3 Results

3.1 Importance of different potential factors on net SOC storage rate

We tested the importance of eight factors that are known to affect net SOC storage rate (N input rate, soil depth, pH, grass/herb ratio, moss cover and clay, silt and sand content). Only two factors significantly affected net SOC storage rate: the N input rate and the grass/herb ratio (Table 1). No interaction between these factors occurred, and the final model yielded a good fit, with an R^2 of 0.99. Therefore further statistical analysis only included N inputs and soil developmental stage as determining factors.

3.2 N availability and N limitation

The PRS-derived N availability in the main rooting zone (0–10 cm) was significantly lower at the low N input sites (E_{NL} and W_{NL}) than at the high N input sites (E_{NH} and W_{NH}) (Fig. 2.A; Table 2). Accordingly, plot-scale biomass, the height of *Cerastium fontanum* and leaf N/P ratios were significantly higher for the high N input sites (Fig. 3; Table 3). Nonetheless, leaf stoichiometry at the high N input sites still indicated N limitation (Fig. 3.A). Soil developmental stage had a marginally significant effect on topsoil N availability, with the M having slightly higher N availability than the E (Fig. 2.A; Table 2). Plant height of *C. fontanum* and total aboveground biomass were significantly higher in M than in E, but leaf stoichiometry did not reveal a significant influence of soil developmental stage (Fig. 3; Table 3).

3.3 N stocks

The effect of chronically elevated N inputs on total N stocks followed contrasting patterns when only topsoil was taken into account or when the total soil profile above the 395 AD ash layer was included. In the topsoil, elevated N inputs increased the total ecosystem N stocks significantly, from 34 to 1080 kg ha^{-1} for E_{NL} and E_{NH} and from 870 to 2200 kg ha^{-1} for W_{NL} and W_{NH}. This N input effect was significant for both plant biomass and soil stocks (Fig. 2.B; Table 2). When the total soil profile was considered (only applicable to W_{NL} and W_{NH}), chronically elevated N inputs significantly increased N stock by 38 %, compared to 121 % in the topsoil (Fig. 2.C; Table 2). This difference was, however, only significant when the N stocks were compared per cumulative soil age (similar to the analysis of the net C storage rate Sect. 2.6 and 2.7; data not shown), and not when the N stock in the bulk soil was considered as a whole (Table 2).

3.4 Soil texture

Following the USDA-NRCS Soil texture classes (USDA 2016), all soils were classified as sand, loamy sand or sandy loam, being very poor in clay (0.0 – 0.2 %), containing 5 – 34 % of silt and being rich to very rich in sand (64 – 94 %). The grain size distribution of W_{NL} and W_{NH} was very similar at all soil depths (Supplementary, Fig. 1), and any differences could be explained by different depths of the 1973 ash layer (shallower at W_{NL}, deeper at W_{NH}; see § 2.1), which has a course structure. The sites with early developmental soils had a similar soil texture to the sites with well-developed soils in the upper layers (0-10 cm), but had a coarser texture in the lower layers (10-30 cm) (Supplementary, Fig. 1), where no soil development had occurred yet.

3.5 Effect of chronically elevated N inputs on C stocks and storage in biomass and topsoil

At the sites with well-developed soils (W_{NL} and W_{NH}), chronically elevated N inputs led to a doubling of the ecosystem biomass plus topsoil C stock, from 17 to 35 ton C ha^{-1} (Fig. 4.A; Table 4). This significant effect of chronically elevated N input on the total C stocks was also visible in almost all individual C stocks: the SOC stock doubled from approximately 10 to about 20 ton C ha^{-1} and the C stock in aboveground vascular plant biomass (“shoots”) increased almost 4 times, from 1.0 to 3.4 ton C ha^{-1} (Fig. 4.A; Table 4). The increase in root C stock in the topsoil of W_{NH} compared to W_{NL} was only marginally significant, but the total root C stock, including deeper roots, was significantly higher at W_{NH} (Fig. 4.A and 4.B; Table 4). The litter C stock remained stable at approximately 1 ton ha^{-1} and the moss C stock decreased from 1 to 0.2 ton ha^{-1} between W_{NL} and W_{NH} (Fig. 4.A, Table 4). The net SOC storage rate in the topsoil of the sites with well-developed soils was 50 % higher under chronically increased N inputs (from 0.30 to 0.44 ton C ha^{-1} yr^{-1}; Fig. 5.B; Table 5).

For the sites in early soil developmental stage (E_{NL} and E_{NH}), the effects of chronically elevated N input were even more pronounced, with all C stocks in biomass and soil significantly higher in E_{NH} than in E_{NL} (Fig. 4.A, Table 4). Biomass C stocks increased from 0.2 to 15.0 ton ha^{-1}, total ecosystem C stock increased by from 1.0 to 22.1 ton ha^{-1} and SOC storage in topsoil increased from 0.02 to 0.28 ton C ha^{-1} yr^{-1}.

Regarding the effect of developmental stage, comparison of the early developmental and well-developed soils over the same timeframe (the last 40 to 50 years) revealed that the total C stock of E_{NL} was only a fraction of W_{NL} (1.0 vs. 30 ton ha^{-1}; Fig. 4.A; Table 4), and that its net SOC storage rate was a mere 9 % of the rate in W_{NL} (Fig. 5.B; Table 5). At the high N inputs sites, on the contrary, all biomass C stocks of E_{NH}, had reached the same level as W_{NH}. This was not the case for the SOC stocks, which, nevertheless, reached half of the W_{NH} stock (Fig. 4.A;

Table 4). In terms of net SOC storage rate, E_{NH} stored half as much SOC as W_{NH} per unit time, and had reached the same rate as W_{NL} (Fig. 5.B; Table 5).

3.6 SOC stocks and storage in the total soil profile

The SOC stocks and storage rates in the total soil profile (since 395 AD) could only be studied at the sites with well-developed soils (W_{NL} and W_{NH}). At both sites, the C stocks and C storage rate decreased significantly with increasing soil depth (Fig. 5; Table 5). However, in ca. 1000 years old soil layers, the C storage reached an equilibrium of 0.12 and 0.16 ton C ha^{-1} yr^{-1} for W_{NL} and W_{NH} respectively. These results also indicate that the chronically elevated N inputs caused a significant increase in net SOC storage of W_{NH} compared to W_{NL}, and that this was maintained throughout the soil profile down to the 395 AD ash layer (Fig. 5.B; Table 5). The effect size of chronically elevated N inputs on net C storage rate decreased with cumulative soil age, as was shown by the significant interaction between N inputs and cumulative soil age (Table 5), until an equilibrium of ca. + 0.04 ton SOC ha^{-1} yr^{-1} (equivalent to +25 %) was reached. When the SOC stocks of W_{NL} and W_{NH} were compared as simple bulk values, integrating the entire soil profile, the differences between W_{NL} and W_{NH} were not statistically significant (Fig. 4.B; Table 4).

Finally, taking the whole measurement depth into account (30 cm for E_{NL} and E_{NH} and up to the 395 AD ash layer for W_{NL} and W_{NH}), the net storage rates of SOC and SON were strongly linked over all treatments, with an average C/N ratio of 12 (Supplementary, Fig. 3).

4 Discussion

4.1 Similarities and dissimilarities between anthropogenic and seabird derived organic N

In this study, a comparison between seabird-affected and unaffected plots was used to test the effects of N enrichment on unmanaged Icelandic grasslands. Seabird-derived N inputs have as main drawback that they differ from the typical anthropogenic N deposition because N is in organic form (Schmidt et al. 2004) and because bird excrements also contain other nutrients (Zwolicki et al. 2013). In our case, the influence of other nutrients is assumed negligible because these ecosystems are strongly N limited (LeBauer & Treseder 2008; see also § 4.2). Despite these potential drawbacks, comparing seabird-affected plots with control plots has different advantages. Firstly, the amount of N inputs is similar to the highest observed atmospheric N deposition (Lamarque et al. 2013). Secondly, and most importantly, the duration of the fertilization is exceptionally long and much longer than what is common for fertilizer addition experiments of which hardly any run long enough to infer long-term effects of anthropogenic N deposition (but see e.g. Hyvonen et al. 2007; Hopkins et al. 2009; Nilsson et al. 2012; Gundersen et al. 2014).

4.2 N availability and N stocks in unmanaged Icelandic grasslands under organic N inputs

The N availability and the total SON stocks were greatly increased in the high N input sites compared to the low N input sites. Interestingly, even though this higher N availability and N stocks at the high N input sites clearly stimulated biomass production and C storage (total aboveground biomass, plant height of *C. fontanum* and ecosystem C stocks were significantly higher), the plant N/P ratios of *C. fontanum* indicated that plant growth remained N limited, in spite of the relatively high chronic N input rates in both E_{NH} and W_{NH} (~47 and ~67 kg N ha^{-1} yr^{-1}, respectively).

Further, the N status was clearly more closely related to the annual seabird-derived N inputs than to soil developmental stage, as the N status of the N enriched grasslands with an early developmental soil was substantially higher than that of the grasslands with a well-developed soil but low N inputs. However, even if the influence of soil development was smaller, there were strong indications that thousands of years of N retention and recycling had (partly) alleviated the N-limitation in W_{NL} and W_{NH} compared to E_{NL} and $E_{NH,}$ respectively, as both the total aboveground biomass and plant height of *C. fontanum* were significantly higher at the sites with well-developed soils. Still, the N availability measured with the PRS probes was only slightly increased in the well-developed soils, while the N stock was substantially higher, indicating that the largest part of the accumulated ecosystem N stock was locked up in undecomposed soil organic matter or in biomass. Increased N inputs to E_{NH} over a short timeframe (26 years), had been sufficient to bring the vegetation N stocks of E_{NH} and W_{NH} at the same level. The SON stocks, however, were still significantly higher in W_{NH}, which shows that the SON stock were not yet in equilibration with the N input rates in E_{NH}.

4.3 Other potential factors affecting SOC storage

Increased N inputs had obviously increased the N stocks and availability of both early developmental soils and well-developed soils, making the sites suitable for studying chronical N input effects on SOC storage. Further, other factors that are known to affect SOC storage under certain circumstances, such as pH (Hobbie et al. 2007; Mueller et al. 2012), moss cover (Post & Kwon 2000; Liu et al. 2015; Vilmundardóttir et al. 2015), clay, silt and sand content (Wiesmeier et al. 2014; Liu et al. 2015) and soil depth (Leblans et al. 2014; Olson & Al-Kaisi 2015), did not significantly affect net SOC storage at our research sites (Table 1). The net SOC storage rate did significantly respond to the grass/herb ratio of the sites. However, this ratio was closely coupled to the effect of the N input rate, which was to be expected, as vegetation development and composition in these N limited systems strongly depend on the N input rate, and the grasses tend to become dominant as the N-inputs increase (Magnússon et al. 2014). The vegetation effect on SOC storage rate was therefore in fact an indirect N input effect. Consequently, we argue that the N input rate is the most important factor in determining SOC storage in these ecosystems, thereby justifying the choice to exclude other factors from our analyses and focusing solely on N inputs and soil developmental stage.

4.4 Net SOC storage in well-developed Icelandic grassland soils under chronically elevated N inputs

4.4.1 Decadal time scale

Our study was conducted on Andosols, which have specific characteristics, including high concentrations of Al, Fe and Si that can e.g. bind SOM in ‘metal-humus’ complexes (Arnalds 2015). Nonetheless, the decadal net SOC storage rate in the well-developed grassland soils (0.30 and 0.44 ton ha^{-1} yr^{-1} at $W_{NL(D)}$ and $W_{NH(D)}$ respectively) corresponded well with the average topsoil SOC storage in a broad range of soil types under previously SOC depleted perennial temperate grasslands (0.33 ton ha-1 y-1; Post & Kwon 2000).

As hypothesized, chronically elevated N inputs stimulated decadal net SOC storage in well-developed soils (i.e. the net SOC storage in the soil layer that was accumulated after 1973). The magnitude of the response is in line with an earlier long-term N addition study on managed grasslands in east Iceland, which reported a > 50 % increase in SOC concentration in the upper 10 cm of the soil after 43 years of fertilization by 120 kg N ha^{-1} yr^{-1} (Gudmundsson et al. 2004). The strong positive effect of chronically elevated N inputs on decadal net SOC storage is in line with the theory that the recent northern C sink is at least partly caused by increasing N deposition (Hudson et al. 1994; Lloyd 1999; Schlesinger 2009).

The net response of decadal SOC storage to chronically elevated N inputs depends on the responses of the individual processes that influence carbon input to and release from the topsoil: (1) net primary productivity (NPP), (2) C partitioning (3) decomposition rate (Mack et al. 2004; Batjes 2014) and (4) the C storage capacity of the soil. (1) Chronically elevated N inputs greatly stimulated NPP in the present study, which agreed with what is generally found in studies that investigate the effects of N inputs on productivity in northern grasslands (Sillen & Dieleman 2012). (2) The root/shoot C partitioning was similar for $W_{NL(D)}$ and $W_{NH(D)}$ (average root/shoot ratios of ~10; data not shown). However, since the total amount of belowground C allocation is potentially also affected by changes in root turnover rates (Aerts et al. 1992; Milchunas et al. 2005), changes in exudation and mycorrhizal activity (Vicca et al. 2012) and changes in microbial C use efficiency (Wieder et al. 2013), the total belowground C inputs cannot be derived from the present data. (3) The response of decomposition to chronically elevated N inputs was not assessed in the present study, but previous studies have reported both positive and negative N input effects on the decomposition rate in the topsoil of northern grasslands. The direction of the response depended on natural background N deposition, N input rate and litter quality (Aerts et al. 2003; Knorr et al. 2005; Hobbie 2008; Zhang et al. 2008). In any case, the increased SOC stocks clearly indicate that the increase in topsoil C input rate surpassed potential increases in decomposition rate in our study. (4) The C storage capacity of the soil determines the maximum amount of C that can be stabilized in the soil, and is strongly dependent on soil texture, where particles ≤20 µm play the most important role in C stabilization (Hassink 1997; Wiesmeier et al. 2014). The soil texture in $W_{NL(D)}$ and $W_{NH(D)}$ was very similar (Supplementary, Fig. 1) and their C storage potential was not yet reached, showing a C deficit of ~50%. Therefore, we assume that the C storage capacity of the soil has not had a significant influence on the C storage in $W_{NL(D)}$ and $W_{NH(D)}$.

4.4.2 Millennial time scale

The total SOC stocks (down to the 395 AD ash layer; 220–280 ton C ha^{-1}) in the present study did not only correspond closely to a previous estimation for Brown Andosols in Iceland (227 ton ha-1; Óskarsson et al. 2004), but were also in line with non-volcanic temperate grassland soils, where estimates range from 197 (Schlesinger 1997) to 236 ton ha^{-1} (Janzen 2004). The observed millennial net SOC storage rates (0.12–0.16 ton C ha^{-1} yr^{-1}) corresponded well with those of deep SOC rich soils in northern regions (0.15–0.30 ton ha-1 yr-1; Trumbore & Harden 1997) and with the long-term SOC storage in temperate grassland ecosystems in China (0.11 ton ha-1 y-1; He & Tang 2008).

As hypothesized, the millennial net SOC storage rate was much lower than the decadal storage rate. This agrees with a recent review study of Matus et al. (2014) that showed a general decrease in net SOC storage rate with depth in Andosols and with the general observation that net SOC storage rates decline when soils approach their mature state (Post & Kwon 2000). However, there was no evidence that the well-developed grassland soils in this study had reached an SOC steady state, as the decline in net SOC storage rate with depth (or with increasing cumulative soil age) stabilized around 1000 years before present and did not decline to zero. Also the potential C storage of $W_{NL(M)}$ and $W_{NH(M)}$ (Supplementary, Fig. 2) showed no indication of C saturation. Both sites with well-developed soils showed a C deficit of ~50% in the deeper soil layers (> 10 cm depth) and even up to the 395 AD ash layer.

The stable C/N ratio of about 12 in the total soil profile of both $W_{NL(M)}$ and $W_{NH(M)}$ suggested that the total SOC stock could continue to increase with elevating N inputs even after millennia of soil development, providing that N can be retained. This was supported by our observation that millennial net SOC storage rate was still increased under chronically elevated N inputs, albeit to a lesser extent than the decadal storage rate (25 % vs. 50 % increase, respectively). The modest increase in SOC storage rate under chronically elevated N inputs, however, was consistent throughout the soil profile and added up to a considerable strengthening of the SOC sink over a long time span while a thicker soil was developed.

4.5 Importance of soil developmental stage for net SOC storage in Icelandic grasslands

Under low natural N inputs, the decadal net SOC storage rate of well-developed soils was substantially higher compared to early developmental soils (i.e. net SOC storage rate $W_{NL}> E_{NL}$), which agrees with an earlier study on a chronosequence of Icelandic Andosols (Vilmundardóttir et al. 2015). The observed increase in SOC storage (~0.30 ton ha^{-1} yr^{-1}) was somewhat higher than the estimates for the transformation from early developmental to more developed Andosols in the abovementioned study (0.1 ton ha^{-1} yr^{-1}), but the latter might be an underestimation as the older soils in their study had not reached their mature state yet (they had been developing for 120 years after a glacial retreat). The increase in net SOC storage rate during soil development is not only an Andosol feature, but is also generally found in other soil orders (e. g. Lichter 1998; Foote & Grogan 2010; Kabala & Zapart 2012; Kalinina et al. 2013) and is caused by centuries of N-accumulation, the stimulation of internal N cycling through biomass and the gradual increase in SOC stock (Vitousek & Reiners 1975; Kirschbaum et al. 2003). Increasing SOC improves the N exchange capacity and the water holding capacity of the soil (Deluca & Boisvenue 2012), thus stimulating plant growth and net SOC storage. In addition, the soil development in W_{NL} has shifted the soil texture to a more fine-grained structure compared to E_{NL} (Supplementary, Fig. 1), especially in the deeper soil layers (> 10 cm soil depth), causing W_{NL} to have a higher potential C saturation (Hassink 1997; Wiesmeier et al. 2014). This more fine-grained structure allows W_{NL} to retain a larger proportion of the incoming C by binding organic matter with clay and silt particles.

We had not anticipated that only 28 years of elevated N inputs at the E_{NH} sites would have created a positive effect on decadal net SOC storage rate similar to that reached at W_{NL} sites after thousands of years of slow N retention and recycling. In fact, the current annual SOC storage rate of E_{NH} most likely surpassed W_{NL}, as it was probably higher in recent years than its average rate calculated since the start of seabird colonization. Indeed, E_{NH} showed a 10-years delay in the establishment of full vegetation cover (and consequently SOC inputs) after the initiation of the allochthonous N inputs (Magnússon & Magnússon 2000). Hence, E_{NH} was likely approaching the decadal net SOC storage rate of W_{NH}. This supposition is supported by the similar biomass C stocks at E_{NH} and W_{NH}, but it can be expected that the development and stabilization of soil processes that regulate net SOC storage will need longer time to come into equilibration with the C input rates (Post & Kwon 2000; Creamer et al. 2011).

If we assume that, with time, the early developmental soils will evolve into similar soils as the soils on the older islands, then the difference in total SOC stock provides an idea of the future ‘SOC gap’ (Kramer & Gleixner 2008). This gap amounted to 220–260 ton SOC ha^{-1} in the present study and will be gradually filled during the process of soil development by the input of organic material at the soil surface and into the rooting zone. Chronically elevated N inputs induced only a slight (20 %) increase in this SOC gap, but did have a large influence on the time frame in which the gap could be filled (by enhancing the net SOC storage rate at E_{NH} by a factor 16). A key question in relation to this is when the apparent SOC gap will saturate. It has been shown that this depends on the status of the SOC stabilizing processes in the subsoil (Fontaine et al. 2007; Rumpel & Kogel-Knabner 2011; Pausch & Kuzyakov 2012; Wiesmeier et al. 2014) and on the frequency of soil disturbance processes (Baldocchi 2008). Until recently, it was assumed that all well-developed soils were SOC-saturated

(Wutzler & Reichstein 2007). However, this supposition has been challenged by various observations of continuously increasing SOC stocks in old (> 1,000 years) undisturbed soils (e.g. Harden et al. 1992; Wardle et al. 1997) and model-based predictions that the equilibration process of SOC stocks could take millennia (Wang & Hsieh 2002). Also our study sites with well-developed soils (W_{NL} and W_{NH}), which considered the past 1,600 years (since a large-scale disturbance before 395 AD), did not seem to have reached SOC saturation yet (see Sect. 4.2.2.).

Considering the large difference in C dynamics that was observed between early developmental and well-developed soils, it is important to bear in mind that many ecosystems are in an intermediate developmental stage, following past disturbances. Therefore, we stress the importance of taking soil developmental stage into account when estimating net SOC storage rates.

5 Conclusion

In our study, the decadal net SOC storage rate of both early developmental and well-developed Icelandic grassland soils was greatly stimulated by chronically elevated organic N inputs, which supported the theory that the increasing northern terrestrial C sink during the past decades has been (partly) caused by increasing anthropogenic N inputs. Importantly, this positive influence of chronic N inputs on the net SOC storage rate persisted at a millennial timescale in the present study, albeit to a smaller extent. This indicates not only that Icelandic grassland soils, if not disturbed, could remain C sinks if the current climate conditions prevail, but also that chronically elevated N inputs could induce a permanent strengthening of this sink.

Author contribution

B.D. Sigurdsson, B. Magnússon, N.I.W. Leblans and I.A. Janssens designed the study and N.I.W. Leblans and B.D. Sigurdsson carried it out. N.I.W. Leblans prepared the manuscript with contributions from all co-authors.

Compliance with Ethical Standards

The authors declare that they have no conflict of interest.

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Figures

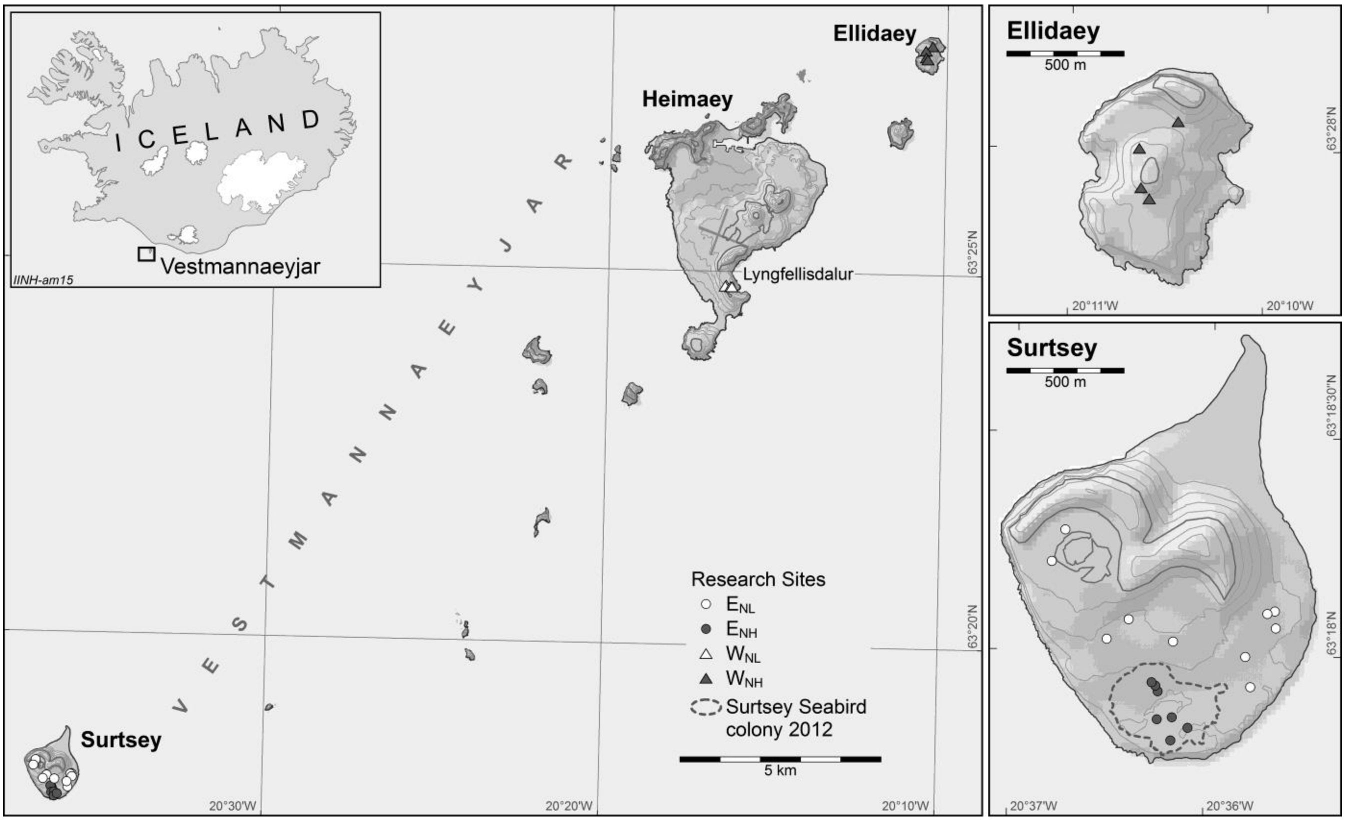

Fig. 1: Topographical map of the research area. (A) Location of the Vestmannaeyjar including the three study islands, Surtsey, Heimaey and Ellidaey at the southwest coast of Iceland. (B) and (C) show the islands Ellidaey and Surstey in more detail. The dotted outline on panel C shows the contours of the seabird colony (that was established anno 1986) in 2012. Dots show the research plots at early soil developmental stage under low (○ = E_{NL}) and high (● = E_{NH}) seabird-derived N inputs. Triangles show the research plots at sites with well-developed soils under low (Δ = W_{NL}) and high (▲ = W_{NH}) seabird-derived N inputs respectively. Map designed by Anette Th. Meier.

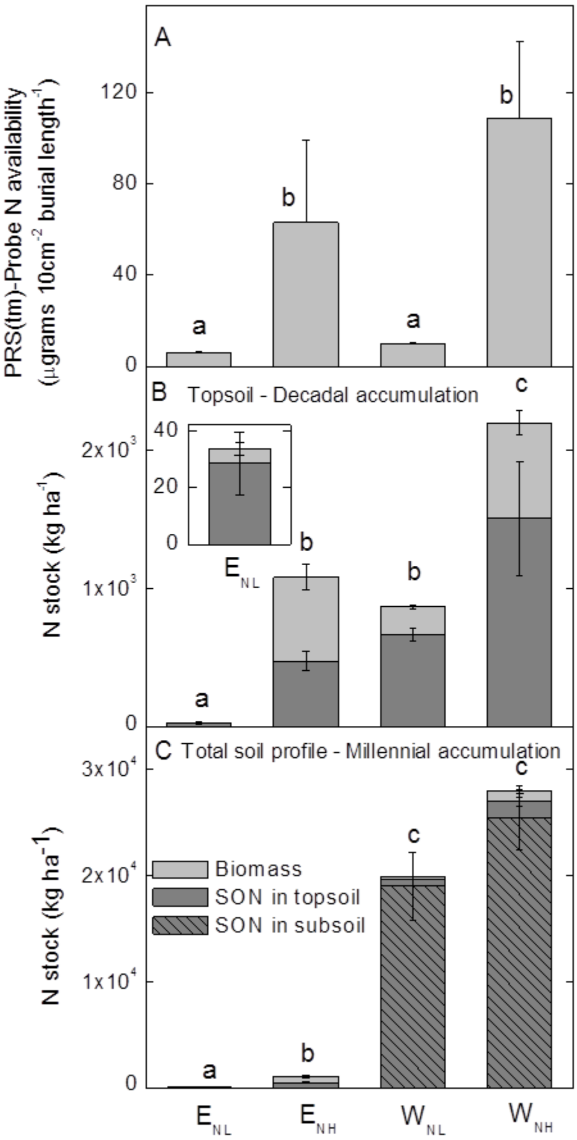

Fig. 2: (A) PRS-probe derived N availability, measured by cation- and anion-exchange membranes that continuously absorb charged ionic species over the burial period, and expressed as soil N flux over time. (B and C) Nitrogen stocks in biomass (shoots + roots) and soil organic nitrogen (SON) in unmanaged Icelandic grasslands under low and high N inputs at an early soil developmental stage (E_{NL} and E_{NH}) and at sites with well-developed soils (W_{NL} and W_{NH}). The SON stocks are shown separately for the topsoil (B; since 1963 for E_{NL} and E_{NH}, above the 1973 ash layer for W_{NL} and W_{NH}) and the total soil profile including the subsoil (C; above the 395 AD ash layer), which corresponds to decadal and millennial SON accumulation respectively. The inserted graph shows the N stock at E_{NL} in detail and is valid for both panels B and C, as E_{NL} had no subsoil. Letters show significant differences in total ecosystem N stocks. Error bars indicate SE's. Statistical details can be found in Table 2.

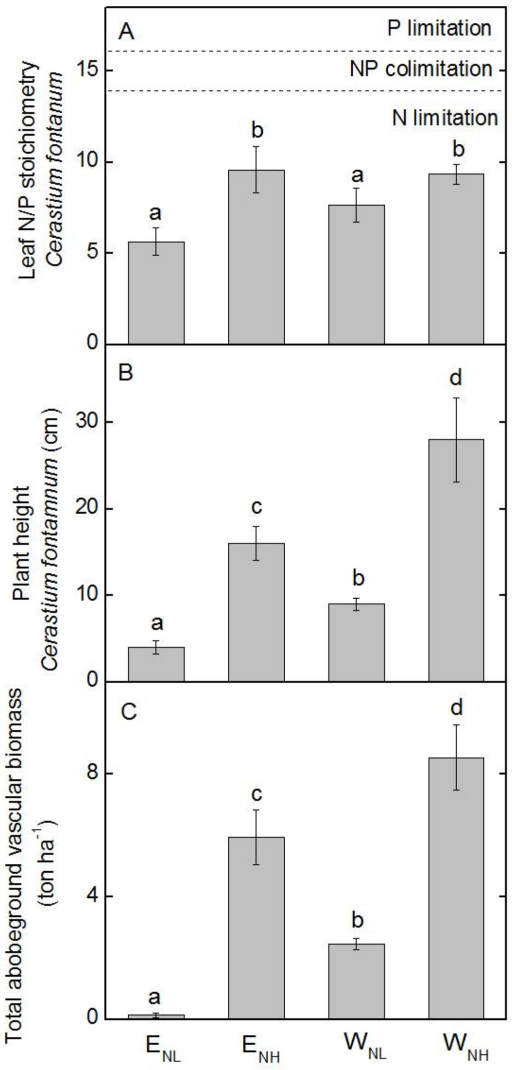

Fig 3: (A) Leaf N/P stoichiometry of *Cerastium fontanum* in mature healthy leaves. Dotted lines show the borders of N limitation (N/P < 14), NP co-limitation (14 < N/P < 16) and P limitation (N/P > 16) for higher plant communities (Aerts & Chapin 2000). (B) Plant height of *C. fontanum*. (C) Total aboveground vascular biomass (monocots and dicots). Different bars shown unmanaged Icelandic grasslands under low and high N inputs at an early soil developmental stage (E_{NL} and E_{NH}) and at sites with well-developed soils (W_{NL} and W_{NH}). Error bars indicate SE's. Letters show significant differences. Further statistical details can be found in Table 3.

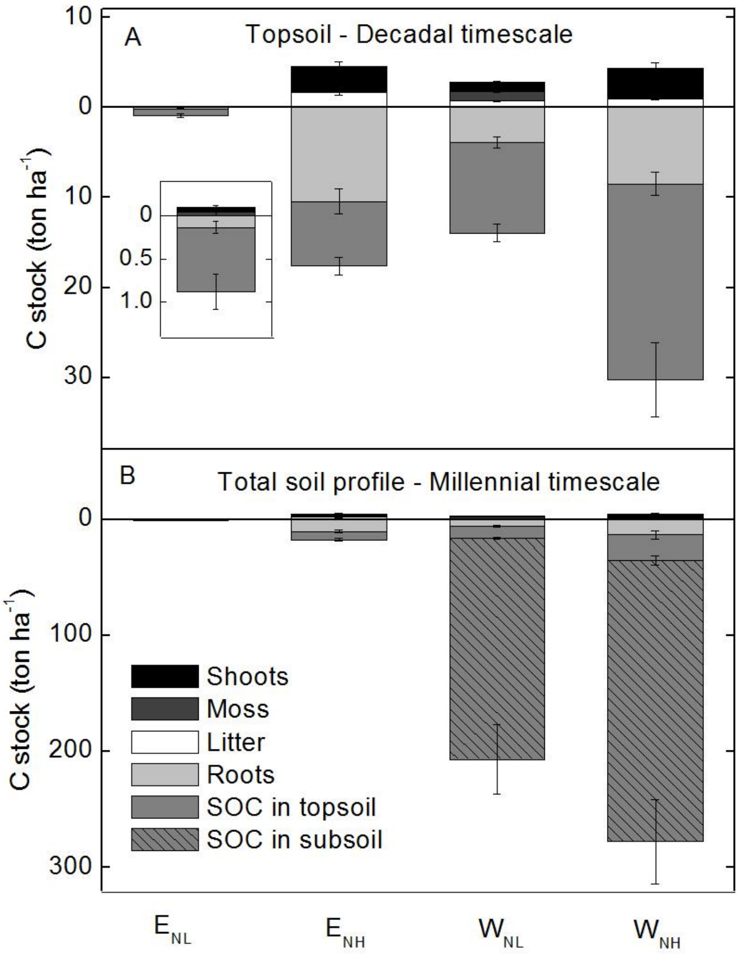

Fig. 4: Carbon stocks in biomass (shoots, moss, litter and roots) and soil organic carbon (SOC) in unmanaged Icelandic grasslands under low and high N inputs at an early soil developmental stage (E_{NL} and E_{NH}) and at sites with well-developed soils (W_{NL} and W_{NH}). The SOC stocks are shown separately for the topsoil (A; since 1963 for E_{NL} and E_{NH}, above the 1973 ash layer for W_{NL} and W_{NH}) and for the total soil profile including the subsoil (B; above the 395 AD ash layer), which corresponds to a decadal and millennial timescale respectively. The inserted graph shows the C stock at E_{NL} in detail and is valid for both panels as E_{NL} had no subsoil. Error bars indicate SE's. Statistical details can be found in Table 4.

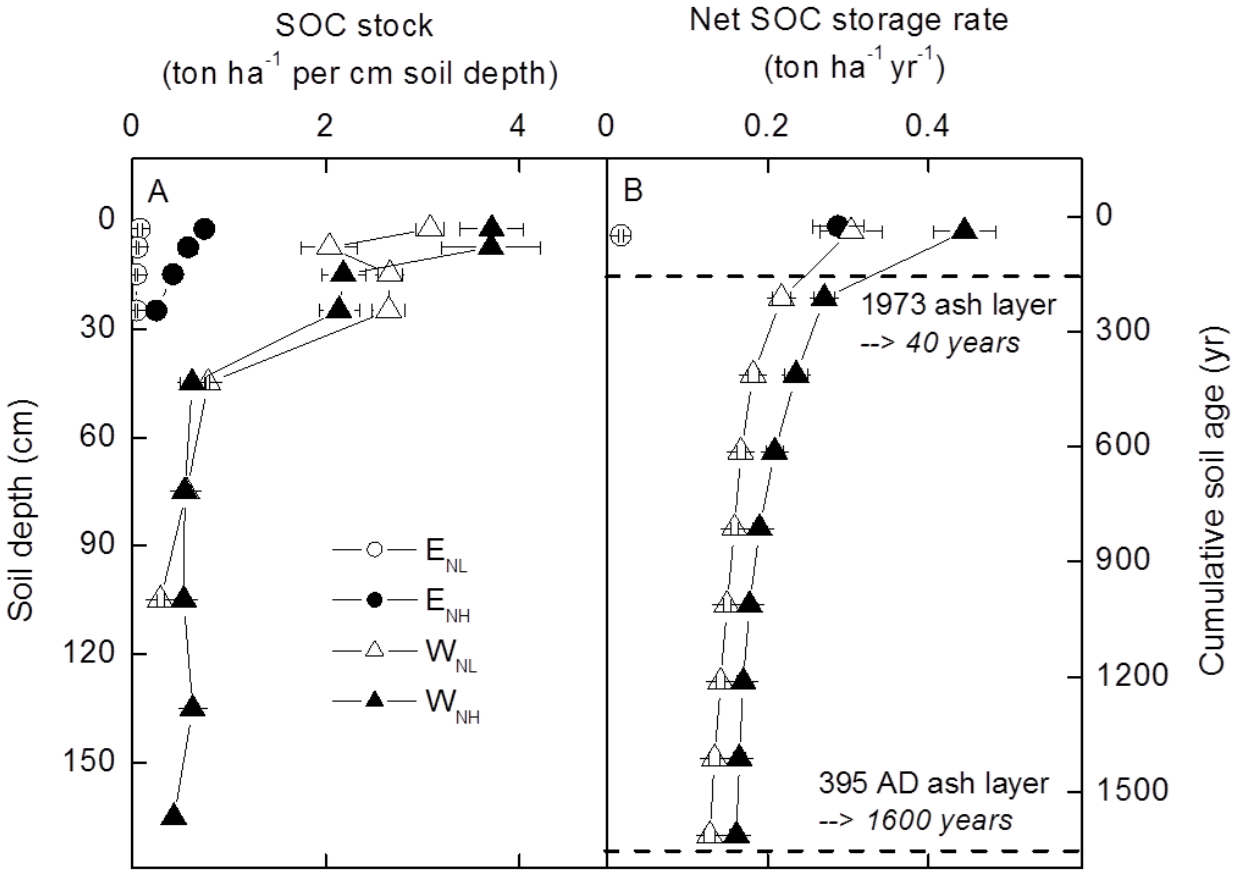

Fig. 5: Soil organic carbon (SOC) stocks and the derived net SOC storage rates in unmanaged Icelandic grasslands under low and high N inputs at an early soil developmental stage (E_{NL} and E_{NH}) and at sites with well-developed soils (W_{NL} and W_{NH}). (A) Depth profile of the SOC stocks per soil layer of one cm, derived from layers of 0–5, 5–10, 10–20, 20–30, 60–90, 90–120, 120–150 and 150–180 cm soil depth. For E_{NL} and E_{NH}, the SOC stocks were measured down to the bedrock or down to 30 cm soil depth, for W_{NL} and W_{NH} up to the 395 AD ash layer. Note the difference in depth of the 395 AD ash layer between W_{NL} and W_{NH}. (B) Net SOC storage rate per cumulative soil age, where soil age was calculated assuming a linear soil accumulation between the 395 AD and 1973 ash layer. The dotted line indicates the 1973 ash layer, which marks the border between the topsoil and the subsoil. The dashed line indicates the 395 AD ash layer, which marks the lower limit of undisturbed soil. Note that E_{NL} and E_{NH} only contain topsoil, as they were still too young to have developed a deep organic soil. Error bars indicate SE's. Statistical details can be found in Tables 4 and 5.

Tables

Table 1: Final model derived from a multiple regression with net SOC storage rate as response variable and different potential influential factors as explanatory variables, which was sequentially simplified by excluding non-significant factors. Presented are the *P* values and slopes of the individual variables, the model R^2 of the final model and the number of samples. Significant source variables ($p < 0.05$) are indicated with an asterisk: *p is 0.05–0.01, **p is 0.01–0.001, ***p < 0.001; n.s. indicates non-significant effects. Uncertainty levels (±) refer to SE's.

Model input variables	**Response variable** *net SOC storage rate (ton ha^{-1} y^{-1})*	
	P	*Slope*
Nitrogen input rate (kg ha^{-1} y^{-1})	***	+10.3 ± 1.1
Ph	n.s.	-0.06 ± 0.05
Grass/Herb ratio	***	+0.28 ± 0.14
Moss cover (%)	n.s.	+0.03 ± 0.02
Clay content (%)	n.s.	-0.18 ± 0.22
Silt content (%)	n.s.	+0.28 ± 0.22
Sand content (%)	n.s.	+0.008 ± 0.223
Soil depth	n.s.	-0.002 ± 0.002
Model R^2	0.99	
N	25	

Table 2: Results of the two-way ANOVA's for N availability and the N stock in biomass, soil (soil organic nitrogen; SON) and the total ecosystem, using N input and soil developmental stage as fixed variables. The topsoil refers to the upper 30 cm or down to the bedrock for E_{NL} and E_{NH} (accumulated since 1963) and to the soil layer above the 1973 ash layer for W_{NL} and W_{NH}. The total soil profile is only applicable to W_{NL} and W_{NH} and refers to the soil profile above the 395 AD ash layer. Significant source variables ($p < 0.05$) are indicated with an asterisk: *p is 0.05–0.01, **p is 0.01–0.001, ***p < 0.001.

		N stock (kg ha^{-1})				
			Topsoil		Total soil profile[b]	
	N availability	Biomass	SON	Total[c]	SON	Total[d]
N input x soil developmental stage						
Df numerator	1	1	1	1	-	-
Df denominator	21	23	23	23	-	-
F-value	0.47	0.58	2.03	0.74	-	-
p-value	0.50	0.45	0.17	0.40	-	-
N input						
Df numerator	na	1	1	1	1	1
Df denominator	na	25	24	25	6	6
F-value	0[a]	68.1	19.60	25.37	2.80	3.54
p-value	***	***	***	***	0.15	0.11
Soil developmental stage						
Df numerator	na	1	1	1	-	-
Df denominator	na	24	24	24	-	-
F-value	101[a]	4.69	21.05	38.47	-	-
p-value	0.06	*	***	***	-	-

[a] Non parametrical Wilcoxon signed rank test: W-value

[b] Only applicable to well-developed soils (W_{NL} and W_{NH})

[c] Cumulation of biomass N and SON in topsoil

[d] Cumulation of biomass N and SON in total soil profile

Table 3: Results of the two-way ANOVA's for leaf N/P stoichiometry of mature healthy leafs of *Cerastium fontanum*, plant height of *C. fontanum* and total aboveground vascular plant biomass using N input and soil developmental stage as fixed variables. Significant source variables ($p < 0.05$) with an asterisk: *p is 0.05–0.01, **p is 0.01–0.001, ***$p < 0.001$.

	Leaf N/P stoichiometry *C. fontanum*	Plant height *C. fontanum*	Total aboveground vascular biomass
N input x soil developmental stage			
Df numerator	1	1	1
Df denominator	12	25	74
F-value	1.12	2.29	0.04
p-value	0.31	0.14	0.85
N input			
Df numerator	1	1	1
Df denominator	14	27	76
F-value	7.16	33.29	75.23
p-value	*	***	***
Soil developmental stage			
Df numerator	1	1	1
Df denominator	13	26	75
F-value	0.15	14.41	12.23
p-value	0.70	***	***

Table 4: Results of the two-way ANOVA's for C stocks in different ecosystem parts, using N input and soil developmental stage as fixed variables. In case of significant interaction, no overall effects of N input or soil developmental stage could be derived and the pairwise differences were tested by post hoc LSD tests or Wilcoxon signed rank tests (lower part of the table). Topsoil C stocks corresponded to C stocks accumulated since 1963 for E_{NL} and E_{NH}, and to C stocks that were accumulated since the deposition of the 1973 ash layer for W_{NL} and W_{NH}. Total soil profile was only applicable to W_{NL} and W_{NH} (E_{NL} and E_{NH} have no subsoils yet), so that the influence of developmental stage could not be tested. It corresponded to the SOC stocks above the 395 AD ash layer. "Shoots" include all aboveground living vascular plant parts; SOC is Soil Organic Carbon. Significant source variables ($p < 0.05$) with respect to ecosystem parts are indicated with an asterisk: *p is 0.05–0.01, **p is 0.01–0.001, ***p < 0.001.

	C stock (ton ha^{-1})								
				Topsoil			Total soil profile[a]		
	Shoots	Moss	Litter	Roots	SOC	Total[b]	Roots	SOC	Total[c]
N input x soil developmental stage									
Df numerator	1	1	1	1	1	1	-	-	-
Df denominator	23	23	22	23	23	23	-	-	-
F-value	0.06	6.58	7.08	6.58	3.17	0.34	-	-	-
p-value	0.81	*	*	*	0.08	0.56	-	-	-
N input									
Df numerator	1	-	-	-	1	1	1	1	1
Df denominator	25	-	-	-	25	25	6	6	6
F-value	33.30	-	-	-	10.15	8.57	11.38	0.68	2.42
p-value	***	-	-	-	**	**	*	0.24	0.17
Soil developmental stage									
Df numerator	Na	-	-	-	1	1	-	-	-
Df denominator	Na	-	-	-	25	25	-	-	-
F-value	111[d]	-	-	-	27.26	34.56	-	-	-
p-value	0.07	-	-	-	***	***	-	-	-
Effect of N input at early soil developmental stage									
Df numerator	-	na	1	na	-	-	-	-	-
Df denominator	-	na	16	na	-	-	-	-	-
F-value	-	35[d]	24.24	0[d]	-	-	-	-	-
p-value	-	0.28	***	***	-	-	-	-	-
Effect of N input at mature soils									
Df numerator	-	1	1	na	-	-	-	-	-
Df denominator	-	6	6	na	-	-	-	-	-
F-value	-	62.69	1.98	1[d]	-	-	-	-	-
p-value	-	***	0.21	0.06	-	-	-	-	-
Effect of soil developmental stage at low N input									
Df numerator	-	na	1	na	-	-	-	-	-
Df denominator	-	na	12	na	-	-	-	-	-
F-value	-	40[d]	261.55	40[d]	-	-	-	-	-
p-value	-	**	***	**	-	-	-	-	-
Effect of soil developmental stage at high N input									
Df numerator	-	na	1	1	-	-	-	-	-
Df denominator	-	na	10	11	-	-	-	-	-
F-value	-	19[d]	1.58	1.07	-	-	-	-	-
p-value	-	0.93	0.23	0.32	-	-	-	-	-

[a] Only applicable to well-developed soils (W_{NL} and W_{NH})

[b] Cumulation of biomass C and SOC in the topsoil

[c] Cumulation of biomass C and SOC in the total soil profile

[d] Non parametrical Wilcoxon signed rank test: W-value

Table 5: Results of the two-way ANOVA's for the net soil organic carbon (SOC) storage rate in the topsoil (decadal net SOC storage rate) and the total soil profile (millennial net SOC storage rate). For the topsoil (left columns), N input and soil developmental stage were used as fixed factors. Topsoil corresponded to the net SOC storage rate since 1963 (down to 30 cm soil depth or down to the bedrock) in the case of E_{NL} and E_{NH}, and to the net SOC storage rate in the soil above the 1973 ash layer for W_{NL} and $W_{NH.}$ For the total soil profile (right columns; only applicable to W_{NL} and W_{NH}), N input and cumulative soil age were used as fixed factors. Total soil profile corresponded to the soil above the 395 AD ash layer. Significant source variables ($p < 0.05$) are indicated with an asterisk: ns. is $p > 0.05$, *p is 0.05–0.01, **p is 0.01–0.001, ***$p < 0.001$.

Net SOC storage rate in the topsoil (decadal storage rate) (ton ha^{-1} yr^{-1})		Net SOC storage rate in the total soil profile (millennial storage rate) (ton ha^{-1} yr^{-1})[a]	
N input x soil developmental stage		N input x cumulative soil age	
Df numerator	1	*Df numerator*	1
Df denominator	23	*Df denominator*	68
F-value	3.39	*F-value*	4.15
p-value	0.08	*p-value*	*
N input		N input	
Df numerator	1	*Df numerator*	1
Df denominator	24	*Df denominator*	68
F-value	47.61	*F-value*	17.00
p-value	***	*p-value*	***
Soil developmental stage		Cumulative soil age	
Df numerator	1	*Df numerator*	1
Df denominator	24	*Df denominator*	68
F-value	33.39	*F-value*	93.22
p-value	***	*p-value*	***

[a] Only applicable to well-developed soils (W_{NL} and W_{NL})