No evidence for differential biomass and mineral content in adult plants grown from dimorphic seeds of *Suaeda aralocaspica*

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PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.650v1 | CC-BY 4.0 Open Access | rec: 1 Dec 2014, publ: 1 Dec 2014

Abstract

Producing two or more types of seeds by a single plant is known as seed heteromorphism. Comparison of seed traits or growth between plants grown from heteromorphic seeds has received considerable attention. However, information is scarce regarding the comparison of mineral content of adult plants from heteromorphic seeds. Here we present biomass and mineral profiles (Nitrogen, Phosphorus, Potassium, Sodium and Chloride) of adult plants grown from dimorphic seeds (non-dormant brown seeds and black seeds with non-deep physiological dormancy) of annual desert halophyte *Suaeda aralocaspica* at different levels of nutrient and salinity. The results showed that, although nutrient and salinity could affect dry weight and mineral content, seed-dimorphic plants of *S. aralocaspica* did not show significant difference under the same experimental conditions. This study is one of the few to compare the physiological responses between seed-heteromorphic plants and suggests that mineral status is exactly consistent with growth performance for these plants.

Keywords: dimorphic seeds, halophyte, mineral content, salinity, seed heteromorphism

Introduction

Plants may produce different types of seeds on a single individual, called seed heteromorphism. Heteromorphic seeds usually differ in their ecological and biological characteristics, such as seed color and shape (Brändel 2007; Song et al. 2008; Ei-Keblawy et al. 2014), germination (Khan et al. 2001; Yao et al. 2010; Aguado et al. 2011), dispersability (Talavera et al. 2010) and soil seed bank (Mandák and Pyšek 2001; Cao et al. 2012). For example, the differences between peripheral and central achenes of annual heterocarpic species *Leontodon longirrostris* result in differences in dispersal and germination (Ruiz de Clavijo 2001).

Carry-over of differential growth and reproduction characteristics has also been reported in many seed-heteromorphic plants, including seedling establishment (Venable and Levin 1985), plant size (Cheplick and Quinn 1982), salt tolerance (Redondo-Gómez et al. 2008; Jiang et al. 2012), phenotypic plasticity (Zhang 1995), competitive ability (Imbert et al. 1997) and reproductive allocation (Sadeh et al. 2009; Lu et al. 2013). Plants grown from heteromorphic seeds generally show different growth characteristics under competitive conditions (Ellison 1987; Imbert et al. 1997). However, under certain conditions, such differences may disappear in the later life-cycle stage. For instance, *Emex spinosa* plants grown from subterranean seeds have larger leaf area and stems than plants grown from aerial achenes in mix culture, but these differences do not exist in monculture when harvested (Weiss 1980).

Growth indices (biomass, plant height and so on) are generally used as the measure to compare seed-heteromorphic plants (Cheplick and Quinn 1982; Ruiz de

Clavijo and Jiménez 1998; Ruiz de Clavijo 2001; Mandák and Pyšek 2005). Few studies compare the physiological responses (antioxidative enzyme activities, chlorophyll content, chlorophyll fluorescence, gas exchange and ion accumulation) between seedlings or adult plants grown from heteromorphic seeds (Redondo-Gómez et al. 2008; Xu et al. 2011; Jiang et al. 2012). These studies show plants grown from different types of seeds maintain differences in physiological responses. As far as we know, there is no record of no physiological differences between plants grown from heteromorphic seeds.

Suaeda aralocaspica (Bunge) Freitag & Schütze (Amaranthaceae), is an annual halophyte restricted to saline-alkaline soils of Gobi desert in central Asia. *Suaeda aralocaspica* germinates from late March to April, flowering occurs from late August to September and fruits mature and disperse in October (Wang et al. 2012). This species produces two types of seeds on a single plant. Brown seeds are oblate, non-dormant and can germinate rapidly at relative high salinity; black seeds are biconvex, have non-deep Type 2 physiological dormancy and germinate slowly even at low salinity (Wang et al. 2008).

In this study, we hypothesized that mineral content of adult plants grown from heteromorphic seeds was consistent with growth performance. In order to test our hypothesis, we designed a single-plant pot experiment to compare the biomass and mineral profiles (Chloride (Cl), Sodium (Na), Nitrogen (N), Phosphorus (P) and Potassium (K)) of adult plants grown from dimorphic seeds of desert halophyte *S. aralocaspica* under different levels of nutrients and salinity.

Materials and methods

Seed collection

In early October 2008, freshly matured fruits of *S. aralocaspica* were collected from a natural population of approximately 600 individuals (44°14′N, 87°44′E; 445 m a.s.l.) in saline desert in the northern part of Xinjiang Province, China (for a detailed description of this site please refer to Wang et al. (2012)). In the laboratory, dry fruits were manually rubbed to detach the seeds enclosed by the membranous pericarps. Then the brown and black seeds were separately stored at 4 °C until used.

Experimental design

The experiment was carried out in a screen house covered with sun shade net (ca. 25 % porosity) at Fukang Desert Ecosystem Observation and Experimental Station of the Chinese Academy of Sciences (44°17′26″N, 87°55′58″E; 460 m a.s.l.).

A randomized block design with six replicates was used. Each block contained 18 pots representing a combination of two seed types (brown and black), three fertilization treatments (low, moderate and high) and three salinity levels (0 as control, low and high). For the fertilization treatment, granular lawn fertilizer (Osmocote 301, Scotts, Marysville, OH, USA; 15N : 11P : 13K : 2Mg) was used as the basic fertilizer and a commercial fertilizer (Peters1, Scotts; 20N : 20P : 20K) as the supplemental nutrient. In the fertilization treatment, each pot received (1) 1.2 g Osmocote 301 and 100 mL Peters1 nutrient solution (0.046 g L^{-1}) once a week (low fertilization), (2) 6 g Osmocote 301 and 100 mL Peters1 nutrient solution (0.23 g L^{-1}) once a week (moderate fertilization) or (3) 12 g Osmocote 301 and 100 mL Peters1 nutrient solution (0.46 g L^{-1}) once a week (high fertilization). Addition of the Peters1 nutrient solution began 3 weeks after sowing. For the salinity treatment, a mixed salt with a 20NaCl : $20Na_2SO_4$: $1NaHCO_3$ (mass ratio) was used. Each pot received (1) 100 mL tapwater (0 g L^{-1}) (control) once a week, (2) 100 mL salt solution (1 g L^{-1}) once a week or (3) 100 mL salt solution (7 g L^{-1}) once a week. To avoid salt shock, 7 g L^{-1} salinity was applied gradually by adding 1 g L^{-1} salinity per week. Adding the salt solution started 4 weeks after planting.

About twenty seeds of brown or black seeds were sown into plastic pots (17 cm deep and 16 cm in diameter) filled with 2000 cm^3 of a vermiculite quartz-sand mix (4:1 v/v) in late April 2009. Germinated seedlings were cultured for nearly 3 weeks and then thinned to one for each container. To reduce variation of initial seedling size, only seedlings of the similar height were used for each seed type. Pots were watered every 2 days, and the same amount of water/ nutrient solution/ salt solution was used for each pot. Five months after sowing, plants for each treatment were harvested.

Measurements and chemical analyses

Shoot samples were oven-dried to a constant weight at 75 °C for 2 days, then weighed by an electronic analytical balance (accuracy: 0.01 g). Oven-dried shoot samples were ground and stored in plastic bags.

Na and Cl were extracted by water extract method. Na content was determined by flame photometry and Cl content by Titrimetric method. For the total contents of N, P and K, 0.5 g samples was transferred into 100 mL digestion flask and 5 ml concentrated H_2SO_4 was added to flask. Then 4 ml H_2O_2 were added twice. The flasks were heated for ten minutes at 360 ^oC and 2 ml of H_2O_2 was added. This step was repeated until the color of the digested samples turned to colorless. N content was determined by distillating method, P content by vanadium molybdate yellow colorimetric method, and K by flame photometry.

Statistical analysis

Three-way ANOVA was used to determine the effects of plant type, salinity and nutrients and their interactions on dry matter yields and mineral concentrations. Tukey's test were performed for multiple comparisons to determine significant (P < 0.05) differences between individual treatments. All data were analyzed using SPSS Version 16.0 (SPSS Inc., Chicago, Illinois, U.S.A).

Results

Effects of different treatments on biomass

There was no significant effect of plant type on biomass (P = 0.634). In addition, there were no significant interactive effects between all three factors. In contrast, there were significant effects of salinity or nutrient levels on biomass (Table 1). Salinity significantly increased the biomass at moderate and high nutrient levels, but not at low nutrient level. Nutrient supplement significantly increased plant biomass. Plants had the highest biomass at high salinity and high nutrient level (Fig. 1).

Effects of different treatments on Cl and Na concentrations

The main effects of plant type on Cl concentration and Na concentration were not significant (Table 1). However, the main effects of salinity on Cl and Na concentrations were significant, i.e. salinity significantly increased Cl concentration at any nutrient levels. In contrast, nutrient supplement significantly decreased Cl concentration. Plants had the highest Cl concentration at high salinity and low nutrient levels, and had higher Na concentration at higher salinity under moderate or high nutrient conditions (Fig. 2). There were significant plant type x salinity, and plant type x nutrient interaction effect on Na concentration, and significant salinity x nutrient interaction effect on Cl concentration.

Effects of different treatments on N, P and K concentrations

The main effects of plant type on N concentration (P = 0.393), P concentration (P = 0.536) and K concentration (P = 0.115) were not significant. However, the main effects of salinity on N and P concentrations, and the main effects of nutrients on N, P and K concentrations were significant (Table 1). There were significant interaction effect of plant type x salinity on P, plant type x nutrient on K, and salinity x nutrient on N, P and K.

Overall, there were no significant differences in N, P and K concentrations between plants from brown seeds and black seeds under the conditions of this experiment. Salinity significantly decreased the N and P concentrations at moderate and high nutrient levels, but not at low nutrient level (Fig. 3).

Discussion

Although differences between plants grown from heteromorphic seeds have been studied extensively (for reviews see Mandák 1997; Imbert 2002), our results are the first in which no significant mineral differences between adult plants grown from heteromorphic seeds have been documented. Dimorphic seeds of *S. aralocaspica* have different mass, dormancy and germination characteristics (Wang et al. 2008). However, the differences are disappear for adult seed-dimorphic plants. This is reflected by not only biomass but also mineral content. So the results of this study support our hypothesis that mineral status is exactly consistent with growth performance of plants grown from heteromorphic seeds.

It is often hypothesized that plants grown from heteromorphic seeds have different competitive ability and stress tolerance. Some seed-heteromorphic species can maintain carry-over differences through the whole life cycle, even in the absence of stress (Weiss 1980; Venable and Levin 1985; Mandák and Pyšek 2005). In contrast, seed-heteromorphic *Crepis sancta* has similar reproductive biomass in the absence of competition (Imbert et al. 1997). There is even no difference in production between mixtures and monocultures of the ray plants of *Dimorphotheca sinuata* (Beneke et al. 1992). It seems that there is no clear fixed pattern for seed-heteromorphic plants.

Growth performance of seed-heteromorphic plants can be related to the physiological measures. Seedlings grown from brown and black seeds of *Suaeda splendens* grow well at 400 mmol L^{-1} NaCl, but seedlings grown from brown seeds does not perform well at low salinity. Growth difference of seed-dimorphic plants can be reflected by chlorophyll fluorescence and gas exchange (Redondo-Gómez et al. 2008). Similarly, for *Suaeda salsa*, Jiang et al. (2012) found that growth status of adult plants derived from dimorphic seeds was related to chlorophyll content and photosynthetic rate. Seedlings grown from yellow seeds of *Atriplex centralasiatica*, which have high salt tolerance, produce greater amounts of NO than do those from brown seeds (Xu et al. 2011). It's clear that physiological differences can reflect growth difference of seed-heteromorphic plants. Furthermore, our results show no difference in growth performance between seed-heteromorphic plants can also be reflected by no difference in mineral content.

Although plants grown from the two seed morphs of *S. aralocaspica* turned out to have similar responses to salinity and nutrient availability under the treatments in this experiment, mineral content are more sensitive to environmental factors than biomass. This is showed by the significant interaction effects between plant type and salinity or plant type and nutrient on different mineral concentrations. In other words, when the availability of salinity and nutrient is beyond the range applied in this study, the possible differences between plants grown from dimorphic seeds will be displayed by mineral concentration firstly.

In conclusion, in this study we documented direct evidence that adult plants grown from dimorphic seeds of *S. aralocaspica* had similar performance in biomass and mineral content under the same environmental conditions. Further researches are necessary to clarify whether the absence of differences in heteromorphic plants is related to other environmental constraints.

Funding

This research was supported by the National Natural Science Foundation of China (31100295) and the West Light Foundation of the Chinese Academy of Sciences (XBBS201101).

Competing Interests

The authors declare there are no competing interests.

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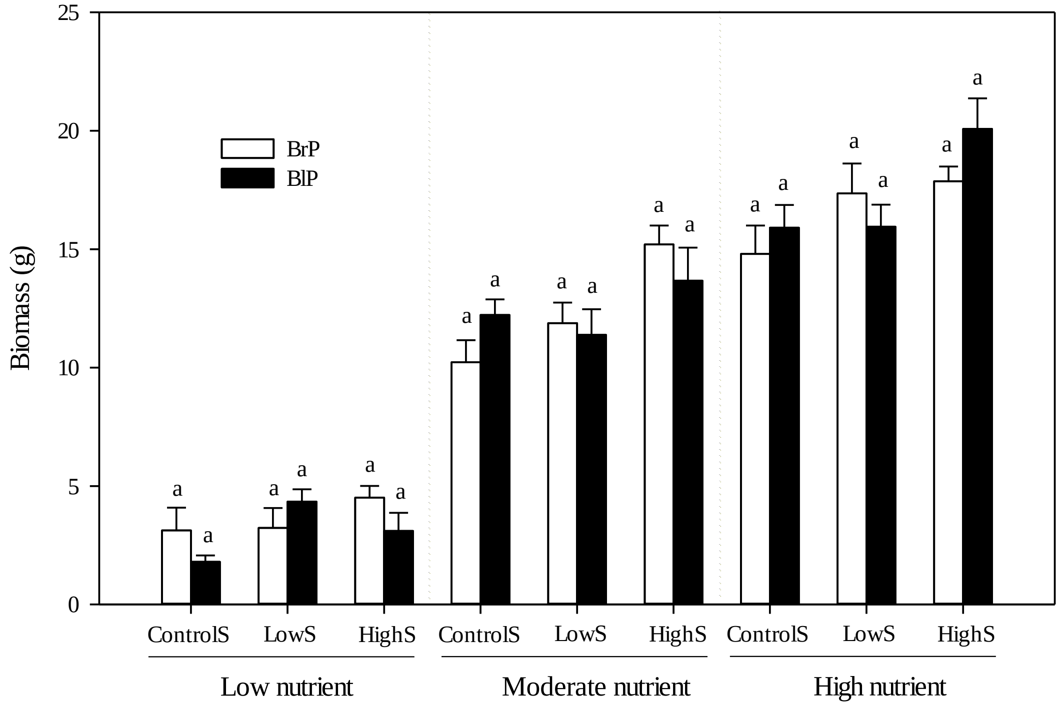
Figure legends

Figure 1. Effects of plant type, salinity and nutrient levels on biomass. BrP: plant grown from brown seed; BlP: plant grown from black seed; ControlS: Control salinity; LowS: Low salinity; HighS: High salinity. Bars with the same letters indicate no significant differences in dry weight of seed-dimorphic plants at a given salinity under a particular nutrient treatment at $P < 0.05$ level.

Figure 2. Effects of plant type, salinity and nutrient levels on Cl and Na concentration. BrP: plant grown from brown seed; BlP: plant grown from black seed; ControlS: Control salinity; LowS: Low salinity; HighS: High salinity. Bars with the same letters indicate no significant differences in Cl or Na concentration of seed-dimorphic plants at a given salinity under a particular nutrient treatment at $P < 0.05$ level.

Figure 3. Effects of plant type, salinity and nutrient levels on N, P and K concentration. BrP: plant grown from brown seed; BlP: plant grown from black seed; ControlS: Control salinity; LowS: Low salinity; HighS: High salinity. Bars with the same letters indicate no significant differences in N, P or K concentration concentration of seed-dimorphic plants at a given salinity under a particular nutrient treatment at $P < 0.05$ level.


25
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Biomass (g)
BrP
BlP
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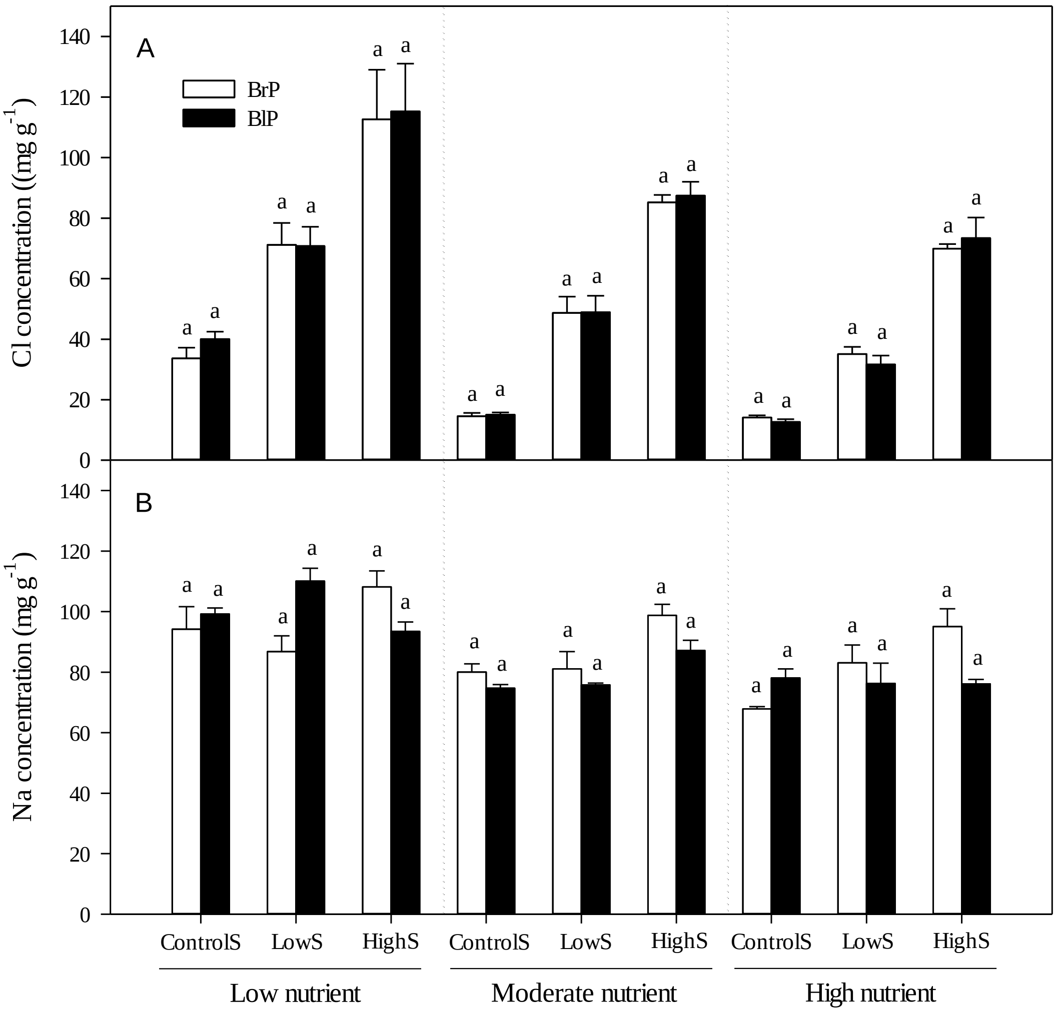
ControlS
LowS
HighS
ControlS
LowS
HighS
ControlS
LowS
HighS
Low nutrient
Moderate nutrient
High nutrient

Fig 1


A
B
Cl concentration ((mg g-1)
Na concentration (mg g-1)
BrP
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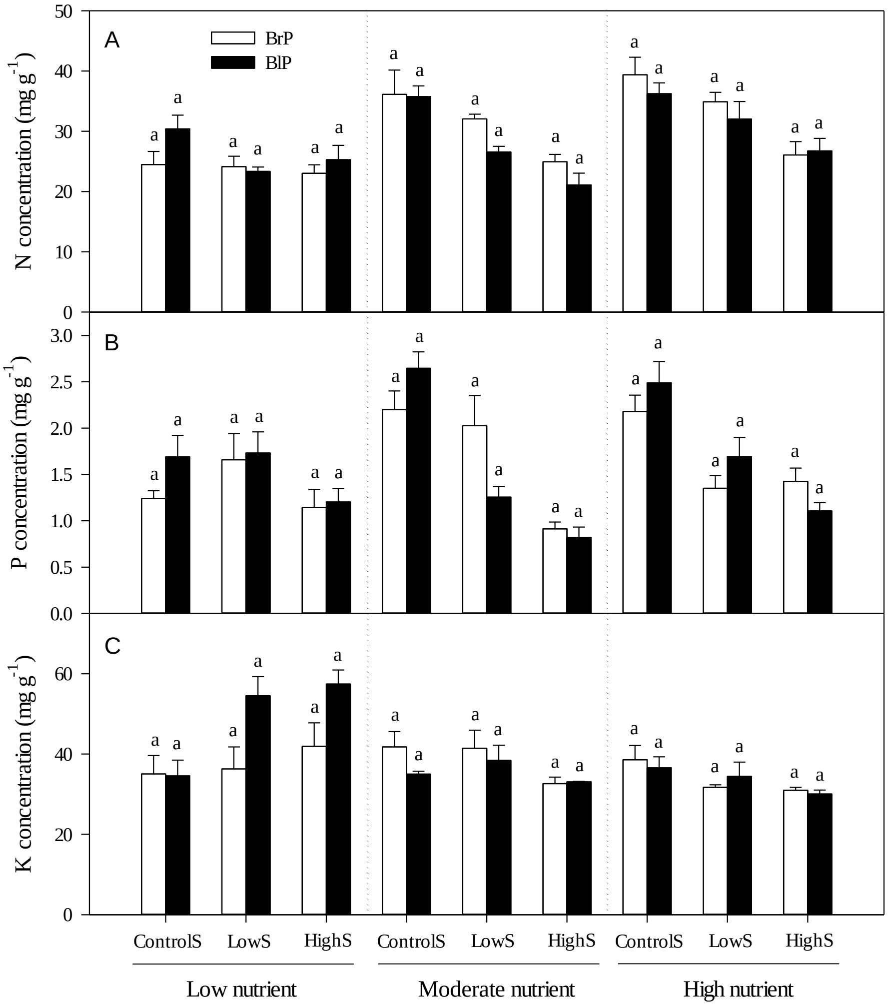
ControlS
LowS
HighS
Low nutrient
Moderate nutrient
High nutrient

Fig 2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N concentration (mg g^{-1})
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ControlS
LowS
HighS
Low nutrient
Moderate nutrient
High nutrient

Fig 3

Table 1 Three-way ANOVA of the effects of plant type, salinity, nutrient levels and their interactions on biomass and mineral content (Cl, Na, N, P and K) of *Suaeda aralocaspica*

Source	Biomass		Cl		Na		N		P		K	
	F-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value
Plant type (PT)	0.04	0.947	0.05	0.825	1.74	0.193	0.741	0.393	0.387	0.536	2.565	0.115
Salt (S)	13.248	<0.001	461.22	<0.001	9.911	<0.001	28.763	<0.001	40.287	<0.001	0.828	0.442
Nutrient (N)	336.666	<0.001	111.618	<0.001	33.885	<0.001	18.992	<0.001	3.209	0.048	11.567	<0.001
PT*S	0.415	0.661	0.293	0.747	9.294	<0.001	1.335	0.272	3.833	0.028	3.035	0.056
PT*N	0.609	0.546	0.883	0.419	3.264	0.046	2.974	0.06	1.275	0.288	6.824	0.002
S*N	1.538	0.198	9.496	<0.001	1.51	0.212	3.474	0.013	7.805	<0.001	6.529	<0.001
PT*S*N	2.347	0.06	0.427	0.788	3.271	0.018	0.612	0.656	2.049	0.1	0.959	0.438