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**Seed germination characteristics of *Polygonum longisetum* (Polygonaceae) with
reference to wildfire**

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ABSTRACT

The seed germination of *Polygonum longisetum* (Polygonaceae) was examined in relation to wildfire, i.e., smoke, soil surface substrates (charcoal and wood ash), litter (fresh and crushed), and water potential because this species is widely distributed as an invasive species and has the ability of smoke-induced seed germination. Previous studies confirm that this species requires light and cold stratification for high seed germination. The effects of seed density and fertilizer on seed germination and seedling growth were also examined. Seed germination was highest at a certain concentration of smoke liquid, while it was lower on woody ash and higher without litter. Since seed germination decreased more with lower water potential, low seed germination on litter and substrates should be induced by water limitation. Seed density and fertilizer did not affect germination rates but changed biomass and shoot height. In conclusion, the effects of smoke liquid on seed germination changed synchronously with the quality and quantity of litter and substrates. These seed characteristics should be advantageous for germination in dry habitats after wildfires and other disturbances.

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litter and soil substrates; *Polygonum longisetum* or *Persicaria longiseta*; smoke-induced seed germination; seedling growth, wildfire

Introduction

Seed germination is promoted by various environmental factors, such as light, temperature (including cold stratification), and water potential (Baskin & Baskin 2014). These environments are changed by litter and substrates burnt by wildfire (Lopez-Almansa 2023). *Polygonum longisetum* Bruijn (syn. *Persicaria longiseta* (Bruijn) Kitag.) (Polygonaceae) is distributed broadly in the warm and cold temperate regions of eastern Asia, including Japan, and often grows in ruderal habitats, such as roadsides and wastelands, as a weedy annual (Ohashi et al. 2021). The seed germination of *P. longisetum* is promoted by light and cold stratification (Tsuyuzaki & Miyoshi 2009) and is less in autumn without cold stratification (Tsuyuzaki 2006). The seeds survived in the soil under 1-3 m thick tephra for more than ten years on an active volcano in Japan, probably because of the stable temperature in the soil (Tsuyuzaki 2010). Furthermore, seed germination of *P. longisetum* is enhanced by smoke exposure with light and cold stratification (Tsuyuzaki & Miyoshi 2009), although Japan is not a fire-prone country, as wildfires burned only 600 ha/yr during 2016 and 2021 (FDMA 2021). *P. longisetum* is a biologically-invasive species in North America and Europe (Celesti-Grapow et al. 2009, Ohashi et al. 2021). The invasiveness of this species seems to be related to the seed germination characteristics. Understanding the seed germination characteristics of this species may provide new insights into seed and fire ecology.

Litter and substrates affect seed germination processes variously via the modification of soil properties (Hao et al. 2012, Egawa & Tsuyuzaki 2013). When wildfire provides smoke, it concurrently alters the quality and quantity of litter, substrates and nutrients (Johnson 1992). Litter distribution patterns, such as patchiness, altered by wildfire affect seed germination (Denham et al. 2009). Therefore, in this work, the effects of fresh and crushed litter on seed germination were investigated. Because charcoal and ash are the major substrates produced from wood by wildfire (Mastrolonardo et al. 2017), the effects of these two substrates were examined. Water potential is altered by wildfire, because of evapotranspiration rate due to altered properties of the ground surface (Tsuyuzaki et al. 2022).

Seedling growth patterns are determined by seed dispersal and the resultant seedling density soon after wildfire (Tsuyuzaki et al. 2014). Wildfire also alters soil properties, namely, nutrient content, and the resultant seedling growth (Reinhart et al. 2016). Therefore, the effects of seedling density and soil

nutrients on seedling growth was also examined. Three hypotheses were checked: 1) smoke liquid at optimal concentration promoted seed germination, 2) Litter and substrates, both of which were altered by wildfire, affected seed germination by modulating water status, and 3) seed density and nutrients (fertilizer) affected seed germination and/or seedling growth (Fenner 2014) after wildfire. The results of this study may explain the characteristics of smoke-related seed germination and the resultant invasiveness of some species after wildfires.

Materials and methods

Seeds were obtained from the Hokkaido University Campus, Sapporo, northern Japan (43°04'N, 141°20'E), a few weeks before starting the experiments. The experiments were conducted from autumn to winter in 2014, 2016, and 2017. When the inflorescences were visually ripe, the seeds were collected by hand every October. The seeds were kept at room temperature until use.

Cold stratification often breaks seed dormancy particularly in temperate regions, although the effect on dormancy break differs among populations (Barga et al. 2017). Therefore, the requirement of cold stratification for the sampled seeds was confirmed at the first year of this research, i.e., in 2014. The seeds were sandwiched between moistened paper towels in a large dish wrapped with a Saran wrap and then exposed to 4°C under dark in an incubator for one month. Seed germination experiments were conducted in petri dishes kept in an incubator or on plastic trays in a greenhouse. The petri dishes were 9 cm in diameter and 15 mm in depth (sterilized e-Schale, Tokyo Glass Kikai Co., Tokyo, Japan). The plastic trays were 17 cm × 12 cm in surface area and 7 cm in depth (Ichigo M-500A, Mineron Kasei Co., Osaka, Japan) with three holes drilled at the bottom. After seed sowing on petri dishes or plastic trays, the number of germinated seeds was counted daily. When no seedlings emerged for more than five days, the experiment ceased, except for 30 days in experiments on water potentials. Seeds were considered as germinated when cotyledons or the radicle appeared. The fluctuations of temperature and light during the experiments were at a cycle of 15/25°C (12 h/2 h) with 12-h light in the incubator, except for the treatment of short (8 h/16 h, light and dark) and long (16 h/8 h) daylengths. Water was supplied by sprinklers nine times every day in the greenhouse without any artificial manipulation of temperature and sunlight.

After the greenhouse experiments ceased, seedlings were harvested and separated into

aboveground and belowground parts and were dried in an incubator at 60°C for over three days. Then, the dry biomass was weighed on each part. Shoot-to-root ratio (S/R ratio) was obtained by (aboveground biomass)/(belowground biomass) for each seedling.

Smoke liquid

To examine hypothesis 1, seed germination percentages were measured at the different concentrations of smoke liquid (Jefferson 2014). Before immersing *P. longisetum* seeds in smoke liquid on November 27 2014, half of the seeds were exposed to cold stratification. Thereafter, 50 seeds were sown on three-layered filter papers (Whatman #1) in each petri dish with five replications for each treatment. The seeds were exposed to smoke liquid (Magic Smoke Home, Logos Ltd., Kyoto, Japan) after dilution with distilled water at 0%, 0.1%, 1.0%, and 5.0%. Therefore, the block design was: (four smoke liquid concentrations) × (presence and absence of cold stratification). Then, the temperature was set at a 15°C/25°C cycle (12 h/12 h) with 12-h light cycle (22 $\mu\text{mol}/\text{m}^2/\text{s}$) between November 27 2014 and January 22 2015.

Substrates and litter

To investigate the effects of litter and substrates on seed germination (hypothesis 2), the experiments started in October 2016 in a greenhouse. Trays were filled with a 5-cm deep layer of vermiculite. Vermiculite was used as substrate ~~soil~~ in all the experiments using trays. For convenience, vermiculite was called soil. Three types of substrates were prepared: vermiculite alone (control) or added either with charcoal or woody ash. Charcoal made of oak (particles 0.5-4 mm diameter) was used (huntsan5L, Ohnishi Forestry, Shiraoi, Japan). Wood ash made of hardwood, dominated by oak, was used as woody ash substrate (MH-01, Chirori Co., Osaka, Japan). In total, 40 seeds were sown in each tray with ten replications per treatment.

The leaf litter of *Rumex obtusifolius* L. was collected from grasslands on the Hokkaido University Campus close to the sites for the seed collection in October 2016, since *R. obtusifolius* was often codominant with *P. longisetum*. After the litter was crushed by hand and finely chopped using a pair of scissors, it was homogenized by passing it through sieves with mesh sizes of 2 mm and 16 mm. After drying the leaf litter in an incubator at 60°C for over three days, the litter was spread out on the

trays covering the soil surfaces. Seeds were sown on litter when soil was covered with litter or on soil when soil was not covered. Therefore, the three levels of litter were prepared, i.e., no litter, crushed litter and fresh litter. Fresh litter was un-crushed and therefore retained somewhat the shape of leaves.

Nutrients, seed density, water potential and daylength

To test hypothesis 3, the effects of seed density and nutrients (fertilizers) on seed germination and seedling growth were examined in 2017. Three levels of seed densities were prepared, i.e., 5, 10, and 20 seeds per tray. Three levels of fertilizer (N: P: K = 6.5: 6: 19, Hyponex, Osaka, Japan) were prepared by diluting the powder at 0% (control), 0.1%, and 0.2%. Before harvesting the seedlings, the normalized difference vegetation index (NDVI) and chlorophyll content of seedlings (SPAD) were measured for each tray. NDVI was measured by a portable chlorophyll fluorometer (FieldScout CM 1000 NDVI, Spectrum Technologies, Inc., Aurora, USA) and SPAD with a portable chlorophyll meter (SPAD-502Plus, Konica Minolta, Tokyo, Japan).

To investigate the effects of water potential on seed germination, five levels of water potential (0.0, -0.1, -1.0, -1.5, and -2.0 MPa) were applied using polyethylene glycol solutions (PEG-6000, Merck, Darmstadt, Germany) (Zheng et al. 2004). In each petri dish, 10 ml of a solution with different water potential was poured on three layers of filter papers. On each dish, 50 seeds were sown and then each dish was wrapped with parafilm. Two photoperiods were used, i.e., day/night cycles of 16 h/8 h and 8 h/16 h. There were five replications for each treatment.

Statistical analysis

All comparisons were conducted by generalized linear models (GLMs) or generalized linear mixed-effects models (GLMMs). All the statistical analyses were performed by R software, version 4.2.0 (R Core Team 2022), with the libraries glmmML (Bröström 2022), lmerTest (Kuznetsova et al. 2017), and lme4 (Bates et al. 2015).

To investigate the effects of cold stratification and smoke liquid concentrations on seed germination, the best model was selected from the four developed GLMs: linear and quadratic regressions with and without cold stratification by Akaike's Information Criteria (AIC). The differences in response variables, i.e., total number of germinated seeds and biomass, were compared

between the control and each treatment by GLMMs. The random effect in all GLMMs was the replication code of the tray or dish. The assumption was that the number of emerged seedlings followed a binomial distribution. GLMMs compared NDVI and SPAD among the treatments with a binomial distribution. Shoot height and biomass followed a Gamma distribution, and the S/R ratio followed a Gaussian distribution.

Results

Effects of smoke substances on seed germination with cold stratification

In the smoke liquid experiments, the number of emerged seedlings per tray ranged from 2 to 29. AICs on the four models were:

One linear line not considering cold stratification (CS) = 258.2

Two linear lines considering CS = 243.2

One quadratic line not considering CS = 255.4

Two quadratic lines considering CS = 237.1

The AIC selected the two quadratic lines considering cold stratification as the best model (Fig. 1). This model selection implied that cold stratification promoted seed germination and that smoke liquid, at optimal concentration, also increased seed germination.

Seed germination on litter and substrates

When comparing the three substrates, the number of emerged seedlings was lowest on woody ash (GLMM, $P < 0.01$) (Table 1), although the differences were small, i.e., 3-4%. Total seedling biomass differed among the substrates (Fig. 2). In particular, total biomass was extremely low on woody ash. Changes in aboveground and belowground biomass on the substrates were similar with changes in total biomass. Aboveground biomass was lowest on ash ($P < 0.01$) and did not differ between control and charcoal ($P = 0.755$). Belowground biomass was lowest on ash ($P < 0.01$) and highest on control substrate ($P < 0.01$). S/R ratio ranged from 0.9 to 11.5 and was highest on ash due to the large reduction in the aboveground biomass. Woody ash and charcoal differently affected seed germination and growth, although both of them were derived by wildfire.

Compared with the substrates, litter tended to reduce seed germination (Table 1). Fresh litter decreased seedling emergence from 36 seedlings (73%) on no litter (control) to 10 seedlings (20%) on fresh litter. Germination on crushed litter was intermediate between control substrate and fresh litter. Biomass ranged from 0.20 mg to 51.7 mg and was highest on control substrate (Fig. 2). These results indicated that crushed litter was more suitable for seed germination than fresh litter but was not suitable for seedling growth. In particular, the seedlings germinated on fresh and crushed litter showed lower aboveground and belowground biomass than controls (aboveground: slope = -0.229, $P < 0.01$ to fresh litter and -0.133, $P < 0.01$ to crushed litter; belowground: slope = -0.451, $P < 0.01$ to fresh litter and -0.157, $P < 0.01$ to crushed litter). These results showed that the presence of litter decreased biomass growth. S/R ratio ranged from 0.1 to 28.7 and was highest on control and fresh litter.

Nutrients, seed density, daylength, and water potential

The seed germination rates did not differ among the fertilizer concentrations (GLMs, $P > 0.05$) (Table 2). However, fertilizer increased shoot height and biomass with increasing fertilizer concentrations. NDVI increased with increasing fertilizer level, while SPAD did not change. Of the five measured biological parameters, seed germination, shoot height, seedling biomass, NDVI, and SPAD, only NDVI increased with increasing seed density (Table 2). These showed that seed density weakly affected seedling performances, although photosynthetic activity (NDVI) increased with seed density.

No seeds germinated at water potentials lower than -1.0 MPa (Fig. 3), regardless of the daylength ($P = 0.582$). When the water potential was zero, seedling emergence was highest, i.e., 24.4 ± 18.1 and 21.6 ± 19.2 under long and short daylengths, respectively. Daylength did not affect seed germination rates ($P = 0.616$).

Discussion

Effects of smoke on seed germination

The quadratic regression analysis indicated that *P. longisetum* had an optimal concentration of smoke liquid for seed germination. *Pyracantha fortuneana* (Hance) Rehder (Rosaceae) and *Osbeckia opipara* C.Y.Wu & C.Chen (Melastomataceae) have optimal concentration of plant-derived smoke solution for

seed germination (Liang et al. 2020). Smoke or its relevant stimuli enhance seed germination for seven out of the 13 species examined in Polygonaceae (Jefferson et al. 2014). From the genus *Polygonum* in the Polygonaceae, three species, *P. aviculare* L., *P. pensylvanicum* L., and *P. persicaria* L. increase seed germination through smoke water treatments (Adkins & Peters 2001). However, it is important to note that the optimal concentrations of smoke water are required for this effect. Post-fire regenerative strategies, characterized by high water use efficiency, are influenced by plant functional traits in Mediterranean shrublands (Saura-Mas & Lloret 2007). This study supports the idea that phylogenetic constraints are associated with germination characteristics, specifically water use efficiency through the manipulation of water potential. Not only seed germination but also multiple growth attributes, such as chlorophyll content, are also enhanced by an optimal concentration of smoke-water for *Carica papaya* L. (Caricaceae) (Chumpookam et al. 2012).

Effects of fire-related substrates and litter

Although both woody ash and charcoal are primarily made from plant materials, mostly wood (Mastrolonardo et al. 2017), they have different effects on seed germination. Crushed litter slightly decreased the seed germination rates, while fresh litter had a greater decrease. Since the seeds were not covered with litter, light interception was not possible. Broad-leaved litter inhibits seed germination by restricting water transfer (Akasaka & Tsuyuzaki 2005, Egawa & Tsuyuzaki 2013). In addition, seedling growth was higher on the soil surface without litter than on fresh and crushed litter. Therefore, litter removed by wildfires plays a positive role in seed germination and seedling growth.

Smoke-related litter and substrates affect seed germination by manipulating soil conditions, such as moisture, on the ground surface. Heat and ash hinder seed germination for a native species, *Daucus carota* L. (Apiaceae), and two invasive species, *Helichrysum foetidum* (L.) Moench (Asteraceae) and *Oenothera glazioviana* Micheli (Onagraceae), following severe wildfires, while they promote the seed germination of *O. glazioviana* after mild wildfires (Fiveiro et al. 2019). Since charcoal and ash affect seed germination differently, the fate of *P. longisetum* seeds is determined by the quality and quantity of litter and substances altered by wildfire intensity.

Nutrients, seed density and water potential in relation to seed germination

No seeds of *P. longisetum* germinated below -1.0 MPa of water potential. Species established in arid habitats tend to germinate even under low water potential. *Ziziphus lotus* (L.) Lam. (Rhamnaceae), which grows in the southern desert of Tunisia, significantly reduces seed germination from almost 100% to less than 10% at -0.8 MPa (Maraghni et al. 2010). Species inhabiting dry habitats in Wales, UK, tend to exhibit high seed germination rates in soil with a moisture potential as low as -1.0 MPa (Evans & Etherington 1990). A meta-analysis suggests that solutions with a pressure lower than -0.8 MPa tend to inhibit the seed germination of both grass and broad-leaved weeds (Singh et al. 2022). Because wildfires often result in dry ground surfaces due to direct solar radiation and the removal of litter in fire-prone regions (Chen et al. 2019, Tsuyuzaki et al. 2022), these conditions are likely to affect seed germination, in dry habitats after wildfire, including the use of smoke as a germination cue.

The daylength did not affect seed germination rates at different water potentials, indicating that water potential determined seed germination regardless of daylength. The seed germination of *Bromus sterilis* L. (Poaceae) occurs even at low water potential, close to the wilting point, but is decreased by exposure to light (Valickova et al. 2017). *P. longisetum* seeds show the highest germination rate when exposed to smoke and cold stratification (Tsuyuzaki & Miyoshi 2009). Seed germination is less affected by seed density and fertilizer because seeds utilize resources stored within them for germination (Fenner 2014). Seedling density and fertilizer concentration affect seedling growth rather than emergence (Fenner & Thompson 2005). In conclusion, smoke and its associated factors, which appear to create optimal conditions, promote the germination of *P. longisetum* seeds and vary with litter and substrates. These changes are likely to have an advantage on seed germination in arid habitats shortly after wildfires and other disturbances.

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Date statement

No potential conflict of interest was reported by the authors.

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Table 1. The numbers of *Polygonum longisetum* seedlings emerged on three substrates, control, charcoal and ash and on three litter types, control, crushed and fresh litter. Each numeral shows mean with standard deviation. GLMMs examine that the numbers of seedlings are different from control to charcoal and ash and from control to fresh and crushed litter. *: significant at $P < 0.01$. NS: not significantly different.

Treatment	Substrate			Litter		
	Control	Charcoal	Ash	Control	Crushed	Fresh
Number of seedlings	30.7 ± 4.1	31.7 ± 3.5	27.7 ± 4.5	36.3 ± 4.8	31.0 ± 4.4	10.1 ± 4.8
GLMM	-2.910*	+0.016 ^{NS}	-1.444*	+0.969*	-0.480*	-2.340*

Table 2. The effects of fertilizer and seed density on the seedling emergence and growth of *Polygonum longisetum*, examined by height, biomass, NDVI, and SPAD. Each numeral shows mean with standard deviation. GLMMs are used to observe the significant difference from no fertilizer and 5 seeds/tray to the other treatments. *: significantly different at $P < 0.01$. NS: not significantly different. The slopes are indicated in the cells of GLMMs when they are significant.

Fertilizer	Density	Germination (%)	Height (cm)	Biomass (mg)	NDVI	SPAD
0	5	24 ± 8	4.7 ± 4.3	28 ± 30	32.5 ± 7.5	35.7 ± 9.5
	10	36 ± 15	6.7 ± 4.4	43 ± 45	38.6 ± 10.3	29.2 ± 7.0
	20	26 ± 17	4.5 ± 3.8	22 ± 33	45.0 ± 8.8	34.7 ± 6.9
1	5	28 ± 30	22.1 ± 6.8	259 ± 145	41.8 ± 13.2	20.3 ± 5.3
	10	32 ± 13	19.8 ± 6.1	213 ± 104	50.4 ± 10.0	22.5 ± 5.2
	20	28 ± 10	18.5 ± 4.7	141 ± 80	48.1 ± 8.4	22.2 ± 3.6
2	5	24 ± 21	25.3 ± 3.9	718 ± 336	46.5 ± 7.9	20.7 ± 5.9
	10	32 ± 14	27.2 ± 4.0	600 ± 298	55.3 ± 8.4	19.6 ± 4.3
	20	29 ± 10	26.0 ± 6.1	500 ± 260	55.5 ± 6.0	19.9 ± 5.3
GLMM	Fertilizer	NS	-0.042*	-0.007*	+0.259*	-0.378*
	Density	NS	NS	NS	+0.017*	NS

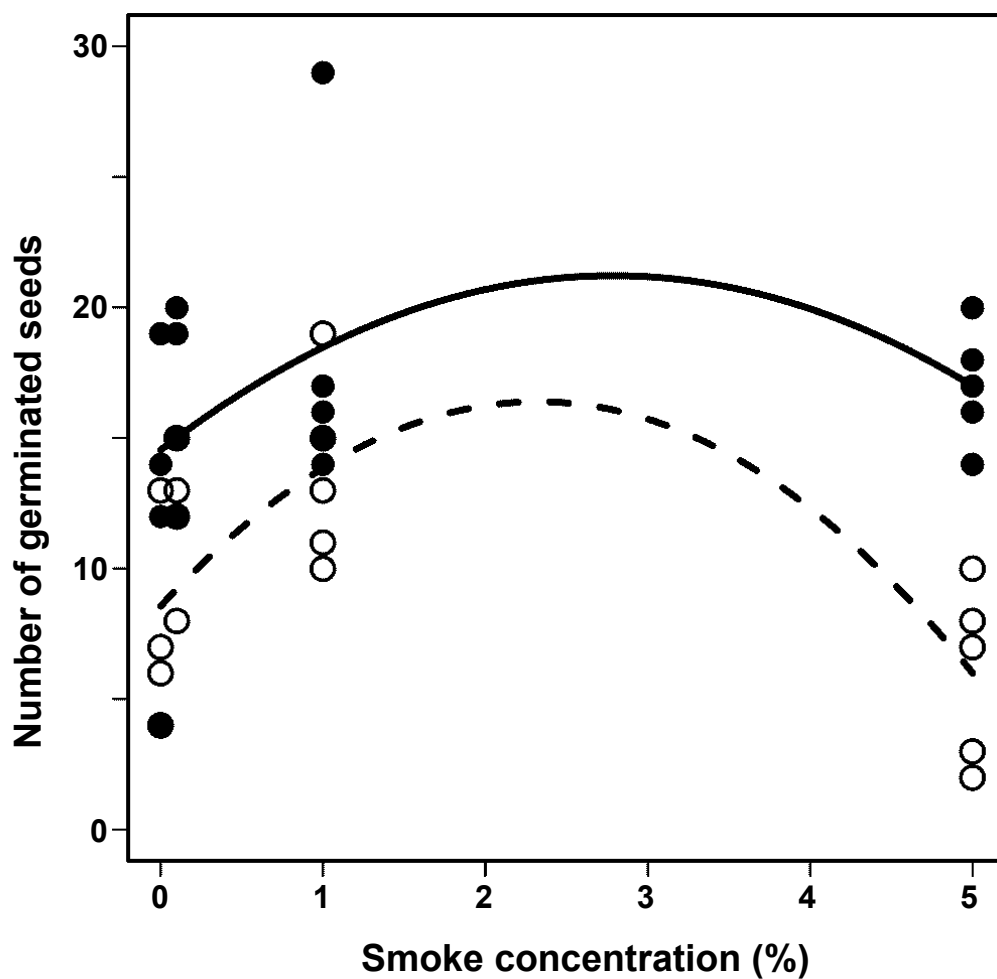


Fig. 1. The numbers of *Polygonum longisetum* seeds (axis y) germinated at different smoke concentrations (axis x). The best fitting lines selected by AIC are $y = -1.451x^2 + 6.741x + 8.553$ with cold stratification shown by solid circles and lines and $y = -0.861x^2 + 4.794x + 14.535$ without cold stratification shown by open circles and interrupted lines.

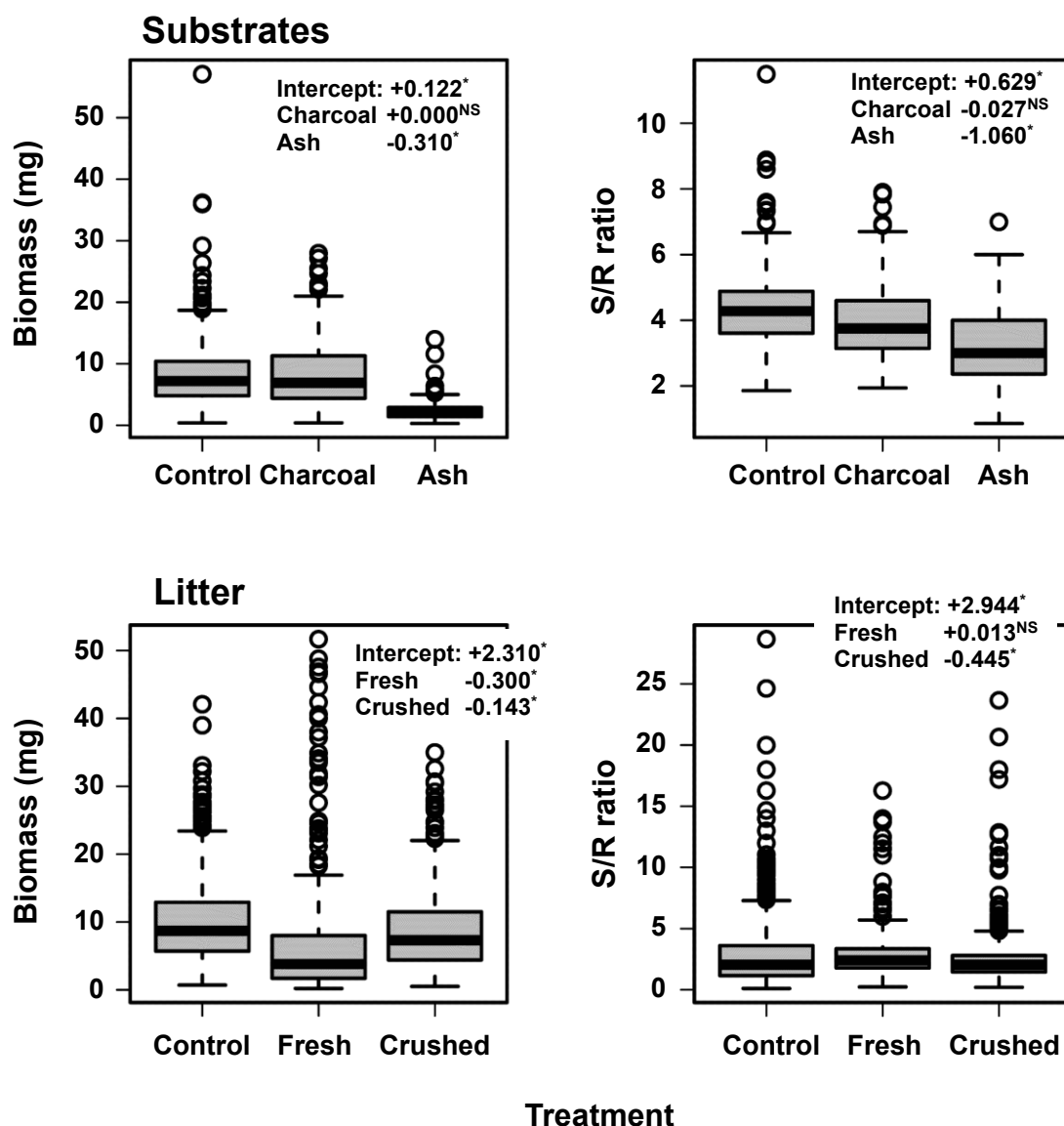


Fig. 2. Effects of substrates (woody ash and charcoal) and fresh and crushed litter on the seedling growths of *Polygonum longisetum*, examined by total biomass and shoot-to-root ratio (S/R ratio). GLMMs are used to confirm the statistical differences. *: significantly different at $P < 0.01$. NS: not significantly different.

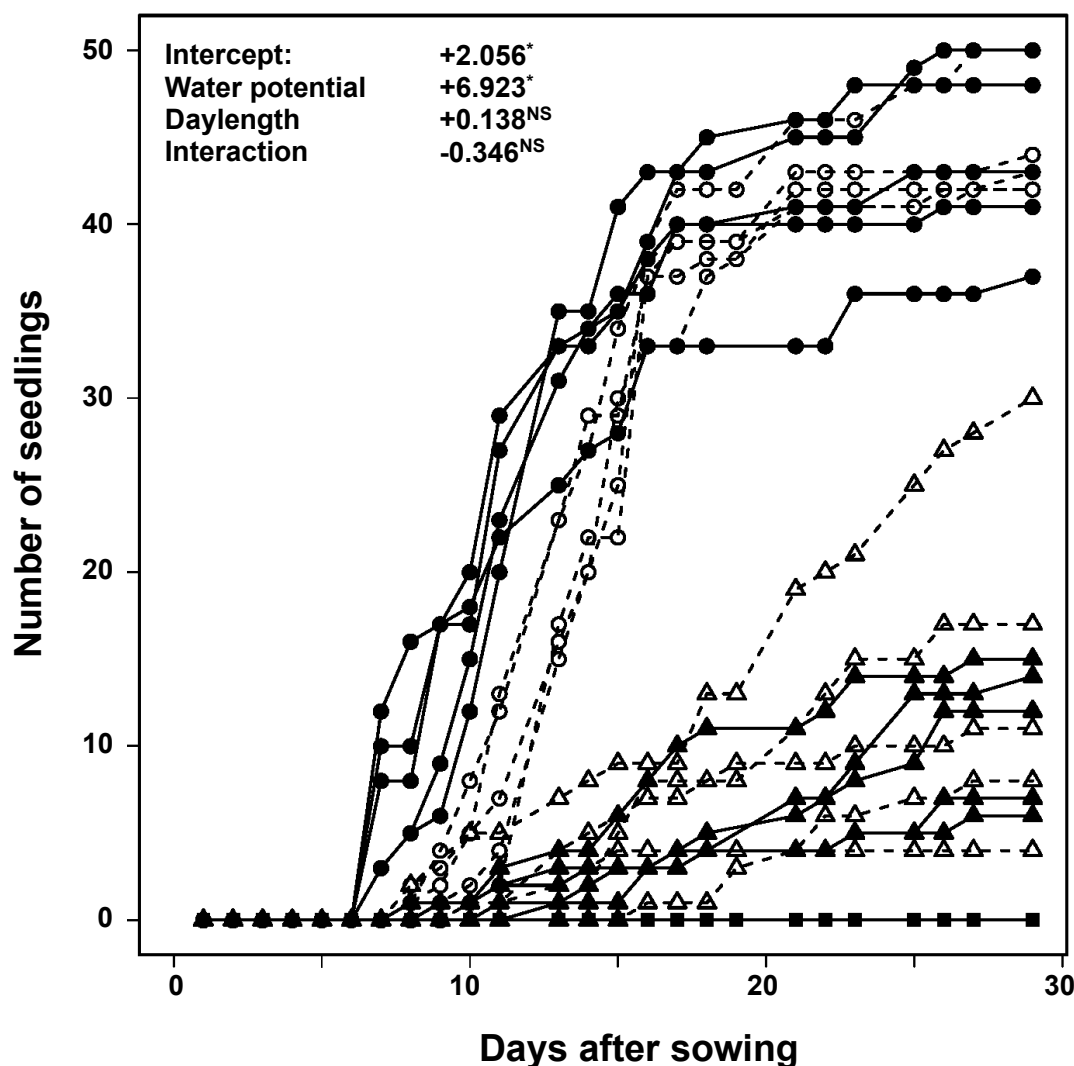


Fig 3. The seed germination patterns of *Polygonum longisetum* at different water potentials, 0.0, -0.5, -1.0, -1.5, and -2.0 MPa under two light conditions, long and short daylengths (16hr/8hr and 8hr/16hr). Closed symbols with solid lines and open symbols with interrupted lines show short and long daylengths, respectively. Circle = 0.0 MPa and triangle = -0.5 MPa. Seed germination at -2.0, -1.5, and -1.0 MPa under any daylengths are shown by squares on the graph because of no germination. *: significant at $P < 0.01$. NS: not significant.