Faculty of Science
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Impact of local heat leakage on vegetation and participation of non-native species

Master thesis

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České Budějovice 2012

Světlíková, P., 2012: Impact of local heat leakage on vegetation and participation of non-native species. Mgr. Thesis, in English. – 61 p., Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic.

Annotation:

Human-induced climate change is a recent phenomenon affecting vegetation structure and functioning. As a proxy of climate change, impact of long-term heat leakage from an underground pipeline on vegetation structure was investigated during a two-year study. Zone above the pipeline was considered as a heat island for non-native, potentially invasive plant species. To test this hypothesis, comparative field and laboratory germination experiments were conducted on closely related invasive and native species. Results of this study provide further evidence that climate change can alter vegetation composition and support invasion of non-native, especially C_4 species.

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V Českých Budějovicích, 13. 12. 2012.

Acknowledgements:

I would like to thank to my supervisors for their valuable advices and help during my master study, to Jakub Těšitel for conducting all analyses in R, to Šuspa and Petr Šmilauer for their advices with vegetation data analyses, to Jan Lechner from Czech Hydrometerological Institute for providing air temperature data, to Jan Wild for providing literature for calibration of dataloggers, and to Josef Holec for providing seeds of *G. ciliata*. I am also grateful to Filip who helped me with my English writing and supported me. Last thanks belong to my family and friends who made all of it possible.

Content:

1. Introduction

Reconstructions of the past millennium climate showed that Earth is anomalously warming from latter 20th century (Mann *et al.* 1999). This observed trend was suggested to exceed the bounds of natural variation (Mann *et al.* 1999; Karl & Trenberth 2003). Although Earth's climate has been naturally fluctuating during its whole history, recent human activities affect it significantly (Karl & Trenberth 2003; Root *et al.* 2005; Huber & Knutti 2011). Greenhouse gases released by human activities to atmosphere trap radiation outgoing from Earth to space and contribute to climate warming. Global mean surface air temperature increased about 0.6 °C per last 30 years (Hansen *et al.* 2006) and this increasing is predicted to continue (Williams *et al.* 2007). By the end of 21st century, mean air temperature can be 1.1–6.4 °C higher relative to the end of 20th century (IPCC 2007). However, Karlén (2005) pointed out that elevated temperature was not observed at several colder areas and that evaluation of short-term observation of temperature can lead to misinterpretations.

Climate warming is related not only to the increase of temperature, but also to droughts (Hoerling *et al.* 2012; Lelieveld *et al.* 2012), rise of sea-level (Meyssignac & Cazenave 2012; Kuhlbrodt & Gregory 2012), more frequent extreme events such as El Niño–Southern Oscillations, floods, tropical cyclones (Easterling 2000; Holmgren *et al.* 2001; Wigley 2009; Cai *et al.* 2012), and temperature anomalies (Hansen *et al.* 2012).

Numerous studies provide evidence corroborating climate warming-induced changes at various levels of biological organization (Walther *et al.* 2002; Root *et al.* 2003; Walther 2003; Parmesan & Yohe 2003; Parmesan 2006; Chen *et al.* 2011; Dossena *et al.* 2012; Lurgi *et al.* 2012). Composition and structure of communities and ecosystems, as well as species' phenology, physiology and distribution are undergoing dramatic changes (Hughes 2000; Sparks *et al.* 2000; Walther *et al.* 2002; Wilson *et al.* 2005; Parmesan 2006; Woodward *et al.* 2010; Saino *et al.* 2010; de Sassi & Tylianakis 2012; Sardans *et al.* 2012; Heath *et al.* 2012). Despite of these dramatic changes across various communities, some communities seem to be unaffected by changing climate (Prach *et al.* 2009; Daniëls *et al.* 2011).

Understanding mechanisms underlying these changes is crucial to predict potential of a species to keep pace with climate warming. According to Gienapp (2008), there are three ways how can a species deal with climate changes in order to avoid far-reaching extinctions. Affected species can stay on original locality and adjust to new conditions by phenotypic plasticity (phenotypic response), adapt to new conditions by evolution (genetic response) or disperse to suitable habitats. Since all three ways are time-dependent and successful establishment is a critical point after dispersion to a new habitat (Savolainen & Pyhäjärvi 2007), the rate of climate change is crucial for species survival.

1.1 Phenotypic plasticity vs. genetic adaptation

A short-term climate warming might be overcome by phenotypic plasticity (Ghalambor *et al.* 2007). However, any response by phenotypic plasticity is limited to a restricted range of temperature. Beyond this range, phenotypic response cannot further mitigate negative effects of enhanced temperature and another response, genetic, has to be involved to prevent detrimental loss of fitness (Gardner *et al.* 2009). Genetic response is based on selection for favourable traits that enable long-term persistence of populations (Donnelly *et al.* 2012). A population persists if the rate of genetic adaptation evolved by selection is at least the same as the rate of climate warming (Bürger & Lynch 1995). Both evolutionary processes are critical for survival of populations facing rapid climate warming and especially for sedentary organisms (i.e. plants) with limited capacity to migrate (Donnelly *et al.* 2012; Zhu *et al.* 2012). However, even species able to respond to these processes are not entirely safe (Bradshaw & Holzapfel 2006), because other drivers of global change as invasions and landscape fragmentation can affect these evolutionary processes (Jump & Penuelas 2005; Hoffmann & Sgrò 2011).

Significant amount of literature has been recently devoted to reveal the occurrence and magnitude of evolutionary processes in species' responses to climate warming. Despite strong evidence available for phenotypic response to climate warming (Menzel *et al.* 2006; Vitasse *et al.* 2010), evidence for genetic response remains scarce (Réale *et al.* 2003; Bradshaw & Holzapfel 2006; Donnelly *et al.* 2012). Lack of latter can be caused by inappropriate method of detection, slow (undetectable) speed of the response, or lagging of the response in time (Gienapp *et al.* 2008; Merilä 2012). Separation of phenotypic and genetic components of the response is difficult, but important step for future predictions of susceptibility of species to climate warming (Gienapp *et al.* 2008; Gardner *et al.* 2009).

1.2 Dispersal to suitable habitats

Third way allowing a species to cope with changing climate is to disperse from original to new habitats. Parmesan (2006) emphasized crucial role of this response during Pleistocene glaciations. Seed dispersal to suitable habitats depends on their availability (establishment limitation) and ability of species to reach suitable habitats (seed limitation) (Clark *et al.* 1998, 2007; Ehrlén & Eriksson 2000). Establishment limitation is determined by number and quality of available habitats (or microsites) suitable for germination, establishment and persistence of species (Clark *et al.* 1998; Juenger & Bergelson 2000). Post-dispersal abiotic factors (temperature, light, moisture, and disturbance), biotic processes (competition from established vegetation, predation, parasitism, and lack of mutualists), seed viability and senescence highly influence the outcome (Maron & Simms 1997; Orrock *et al.* 2006; Clark *et al.* 2007; Bruun 2010). On the other hand, seed limitation is determined by number of produced seeds (source limitation) and by their dispersal ability (dispersal limitation) (Clark *et al.* 2007).

Relative importance of both these limitations in shaping composition, abundance and distribution of plant species on a local-scale have been thoroughly discussed (Eriksson & Ehrlén 1992; Clark *et al.* 2007). Some studies demonstrated seed limitation (Primack & Miao 1992; Dalling *et al.* 2002; Rey *et al.* 2005; Ehrlén *et al.* 2006; Marsico & Hellmann 2009), while other reported establishment limitation (Crawley 1990; Turnbull *et al.* 1999; Hubbell 1999; Pearson *et al.* 2002). Recently, it was shown that both limitations can participate at once (Eriksson & Ehrlén 1992; Honnay *et al.* 1999; Ehrlén & Eriksson 2000; Dalling *et al.* 2002; Clark *et al.* 2007; Emery *et al.* 2009; Dullinger & Hu 2011) and that establishment limitation may be more important than ever thought (Tilman 1997; Zobel & Kalamees 2005; Zeiter *et al.* 2006).

1.3 Vulnerability of species to climate change

Several species have been shown to be unable to track changing climate (Primack & Miao 1992; Marsico & Hellmann 2009; Van der Veken *et al.* 2012; Zhu *et al.* 2012), which can, in turn, put them to high risk of extinction (Root *et al.* 2003; Thomas *et al.* 2004; Parmesan 2006). According to Broennimann *et al.* (2006), species' vulnerability is determined by its geographical distribution, niche breadth or proximity to barrier preventing migration, and can be *a priori* estimated. However, due to paucity of available knowledge about responses on species level, it is very challenging to assess vulnerability of individual species (Holt 1990; Broennimann *et al.* 2006; Hoffmann & Sgrò 2011). Incorporating this knowledge to new conservation strategies is critical to prevent or minimize extinction and loss of biodiversity (Root & Schneider 2006; Lawler *et al.* 2010).

One of proposed strategies (called assisted migration or colonization) suggests introduction of potentially endangered species beyond their historical range so that they will be able to survive under future climate (McLachlan *et al.* 2007). An outburst of controversy has arisen about the implementation of this strategy, because it carries, among others, risk of introducing potentially invasive species to new areas (Hoegh-Guldberg *et al.* 2008; Loss *et al.* 2011). Hoegh-Guldberg *et al.* (2008) emphasized the importance of thoroughly understanding the strategy before its implementation and botanical gardens were suggested as an ideal place for the strategy testing (Primack & Miller-Rushing 2009).

1.4 Experimental approaches for testing limitations

The most common approach testing relative importance of seed and establishment limitations is to perform a seed addition experiment (Turnbull *et al*. 2000, 2005; Clark *et al*. 2007, Table 1). According to Turnbull et al. (2000), there are two types of seed addition experiments. Seeds are either added to areas where the species normally grow (seed augmentation) or they are introduced to new areas (seed introduction). Evaluation of such experiments is hampered by drawbacks (Turnbull *et al.* 2000; Clark *et al.* 2007) and several authors pointed out misleading results coming from short-term observations (Zobel & Kalamees 2005; Ehrlén *et al.* 2006; Gaston 2009).

Seed introduction can be also implemented in studies predicting the potential of plant species to colonize new ranges (Mack 1996). Only small number of studies introduced seeds of target species beyond their distribution range for this purpose and most of them examined only a single species in a short-term experiment (Levin & Clay 1984; Andersen *et al.* 1985; Prince & Carter 1985; Van der Veken *et al.* 2007; Van der Veken *et al.* 2012). Mack (1996) suggested that combining seed introduction approach with other approaches, such as field manipulations of environment or comparison between closely-related congeners, will provide finest prediction. Comparisons between two congeneric species (non-native and native) can reveal shared traits and, more importantly, different traits which can be responsible for different performance in a new range (Mack 1996). This combined comparative approach has been incorporated only in few studies. Notably, it was recently implemented by Sheppard *et al.* (2012) to compare performance of non-native and native meadow species under conditions simulating climate change (extreme events and warming).

1.5 Objectives of study

Here, I take advantage of existence of underground steam pipeline system in České Budějovice (Czech Republic) to disentangle effects of soil temperature increase on vegetation and particularly on the occurrence of invasive species. During the two-year period, comparative approach was used to assess vegetation and temperature differences between the zone with heat leakage and heat non-affected control zone. To test if the pipeline zone can serve as a heat island where non-native thermophilic species can recruit and establish, invasive species were sown in a field experiment and compared to their non-invasive relatives.

Based on knowledge gained during my bachelor study of vegetation above the pipelines, I have decided to test following hypotheses:

- vegetation above the pipeline differs from surrounding vegetation
- short-lived plant species are widely represented above the pipeline
- non-native species are able to recruit and establish above the pipeline more easily than outside it

2. Materials and Methods

2.1 Study sites and experimental design

The experiment was carried out at three sites (designated as site 1, 2 and 3 hereafter) in České Budějovice (Czech Republic) from 25 June 2010 till 20 June 2012. All the study sites are located near each other (48°58'24"N, 14°27'28"E, 380 m a.s.l.) above the underground steam pipeline in the local park (Stromovka). The pipeline is formed by two pipes of different diameter size. The larger pipe conveys steam of approximately 180 °C and the smaller pipe conveys hot water of approximately 70 °C. The pipeline was built between the years 1982-1985 and is deposited on gravel ballast around 1.5 m underground.

Although all three sites are covered by mown lawns, only sites 1 and 2 are cut regularly and at the same time. Cutting starts in April or May and continues with a month period to November. Site 3 is cut from August 2011 only few times per a season. Fifteen 1×1 m plots were delimited at each site in three zones: five plots were established in the middle of the zone above steam pipeline (zone A), five plots were located half a meter from them (zone B, outside the pipeline) and five remaining plots were located fifteen meters apart from previous plots (zone C, Fig. 1). Zone A was determined using maps of the pipeline (provided by Teplárny České Budějovice, a.s.) and vegetation differences. The distance between each two adjacent plots in each of the three zones was three meters (Fig. 1). Percentage cover of all vascular plants was estimated in plots every two weeks in spring and summer and in the rest of the year monthly if possible (e.g. not covered by snow). Nomenclature of plant taxa, except *Galinsoga ciliata*, was used according to Kubát *et al.* (2002).

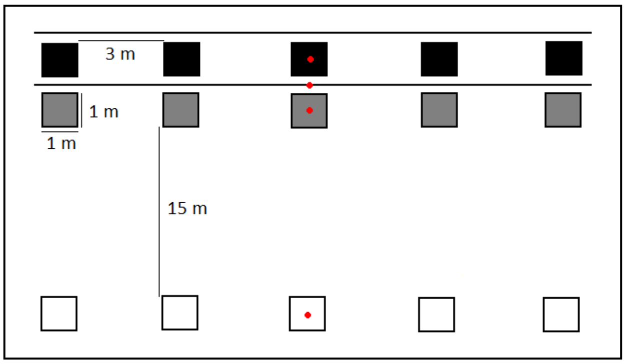

Fig. 1. Design of plots at each site. Zone A is represented by black squares, zone B by grey squares and zone C by white squares. Red points symbolize the locations of Minikin dataloggers.

Unfortunately, five C-plots at the second site were destroyed (covered by soil) in September 2010. Since data were sampled only five times from these plots, they were completely eliminated from any subsequent analyses to prevent potential biases.

2.2 Soil temperature and moisture measurements

Soil temperature was measured by twelve Minikin (EMS) and four TMS first generation (TOMST®) datalogger devices with an accuracy of 0.2 and 0.5 °C, respectively. At each site, four Minikin devices were buried a few centimeters underground as described below. The first was placed into the middle of the zone above steam pipeline (zone A), the second was placed into the middle of intermediate plot outside the zone (zone B), the third was placed between these two devices (in the transition zone, zone A-B) and the last was laid into the middle of the most remote plot (zone C, Fig. 1). Minikin dataloggers recorded temperature every 30 minutes from 7 July 2010 till 14 August 2012. TMS dataloggers were placed near already deposited Minikin devices to the second site and were primarily used to measure soil moisture every 30 minutes from 1 December 2011 till 30 November 2012.

Downloaded soil moisture data, expressed in arbitrary units, were converted to the percentage of volumetric water content using a calibration curve. Calibration of devices was done according to instructions in Vlček (2010). The following regression equation was obtained: $y = 0.00034x - 0.20754$, and the coefficient of determination $R^2 = 0.99848$ (Suppl. Mat. 1).

2.3 Sowing experiments

The ability of five chosen closely related species to germinate, survive and potentially reproduce in the zone above steam pipeline was examined by a field experiment. Simultaneously, a laboratory germination experiment was carried out to provide background knowledge about the influence of various temperatures on species germination. Phylogenetically closely related species from two distinct clades of Asteraceae were chosen for comparisons. Two native *Senecio* species (*S. jacobaea* and *S. viscosus*) were compared to non-native *Senecio inaequidens*, and non-native *Tridax procumbens* was compared to naturalized non-native *Galinsoga ciliata.*

2.3.1 Studied species

Senecio inaequidens DC. (narrow-leaved ragwort, South African ragwort)

S. i. is a perennial herb reaching up to 1 m with original distribution in mountainous regions of Lesotho and South Africa (Hilliard 1977; Monty *et al.* 2010; Lachmuth *et al.* 2010). In its native range, it colonizes primary habitats such as rocky slopes, outcrops, and gravel banks, but it also grows in disturbed areas such as vineyards, roadsides, coastal dunes and heavily grazed grasses (Lafuma & Maurice 2007; Bossdorf *et al.* 2008; Caño *et al.* 2009).

By the end of the 19th century, the species was introduced into Europe. As all centers of early introduction (Bremen, Calais, Lüttich, Verona, Mazamet) were associated with wool trade, sheep wool is considered to be a source of seeds (Ernst 1998; EPPO 2006). Until 1950s, *S. inaequidens* was found only around existing wool-processing factories (Ernst 1998), but since then it has rapidly spread throughout western, central and southern Europe (Monty & Mahy 2010). In 1997, the species firstly reached the Czech Republic (Pyšek *et al.* 2012). It is assumed that a significant amount of seeds might be transported on long distances in the profile of tires (Heger & Böhmer 2006).

Not only human unintended assistance, but also several life-traits allowed *S. inaequidens* to become troublesome invasive species in Europe (Schmitz & Werner 2001). For example, its long flowering period enables the species to produce more than 100,000 achenes per an individual a year (Böhmer *et al.* 2001; López-García & Maillet 2005; Heger & Böhmer 2006). High outcrossing rate resulting from self-incompatibility can be other contributing factor explaining its high seed set (Vanparys 2011).

Although *S. inaequidens* invades mainly ruderal habitats in its secondary range, it has recently occurred in natural communities as dry grasslands (Bossdor*f et a*l. 2008), pine forests and coastal dunes (Brunel 2003; Lachmuth *et al.* 2010). Noted as noxious weed at Mediterranean pastures, it can poison livestock because of its substantial level of pyrrolizidine alkaloids (Dimande *et al.* 2007). On the other hand, avoidance by grazing animals acts as competitive advantage further promoting its invasive features (Bossdorf *et al.* 2008).

Senecio jacobaea **L. (ragwort, tansy ragwort)**

S. j. is a monocarpic perennial (rarely annual) commonly reaching about 50 cm in height. The plant usually forms a rosette during the first year of life and flowers in the following season (Wardle 1987; Parsons & Cuthbertson 2001). It reproduces both sexually and vegetatively (Poole & Cairns 1940). Because of the self-incompatibility of the species, sexual reproduction fully depends on insect pollinators (Andersson 2001). Produced achenes are distinguishable to two morphologic groups possessing different germination and dispersal abilities (McEvoy 1984; McEvoy & Cox 1987).

According to Sharrow *et al.* (1988), ragwort is native to Europe and western Asia (from Norway to Asia Minor and from Great Britain to Siberia) where it naturally colonizes open grasses, woodland communities and sand dunes, and can be also found in many human disturbed habitats (Harper & Wood 1957; Bain 1991). Although it is able to inhabit a broad range of habitats (Harper & Wood 1957), it prefers the mesic ones (Bain 1991). Nowadays, it is regarded as a pasture weed in many temperate areas (Australia, New Zealand, South Africa, South and North Americas) into which it was introduced in the 19th century (Wardle *et al.* 1995; Parsons & Cuthbertson 2001). As it contains pyrrolizidine alkaloids similarly as *S. inaequidens*, a lot of attention has been paid to the control of this species (McEvoy *et al.* 1989; Bain 1991; Crider 2011).

Senecio viscosus **L. (sticky groundsel, sticky ragwort)**

S. v. is an annual early-flowering herb capable of self-fertilization (Warwick *et al.* 1978). It was primarily distributed around temperate Asia and Europe (except the northern part) across various habitats containing rocky slopes, open forest, sandy or gravelly places, and wastelands (Hegi 1987). Later, the species was introduced to northern Europe, United States, and Canada where it become naturalized.

Tridax procumbens **L. (coat button, tridax daisy)**

T. p. is an annual or short-lived perennial originally native to Central and tropical South America and the West Indies, but now widespread all over the tropical and semi-tropical latitudes (West Africa, Southeast Asia, and Philippines). In the secondary range, the species predominantly colonizes arable land, but it also grows along railroads, roadsides, waste grounds, dikes, riverbanks, dunes and meadows. Subsequent dispersal to colder latitudes may be limited by high temperature required for germination (Chauhan & Johnson 2008).

As a successful weed, coat button produces high amount of seeds or rapidly spreading stems (Baker 1965) and negatively affects surrounding vegetation by allelopathy (Holm *et al.* 1997). Its effects notably decrease crop yields and so it is listed as a noxious weed that has to be regulated (USDA 2010). Ipou *et al.* (2011) suggested annual leguminous plants to control the species in cotton culture in the North of Ivory Coast.

Despite the previous facts, *T. procumbens* is also used as a medicinal plant with antibacterial activity and capacity to heal wounds and stop bleeding (Samy *et al.* 1999; Kumar *et al.* 2007).

***Galinsoga ciliata* Raf. (hairy galinsoga, shaggy soldier)**

G. c. is an annual herb closely related to *T. procumbens* (Watson *et al.* 1991; Bergquist *et al.* 1992) and resembles it in many characteristics such as appearance, place of origin and reproductive traits.

Original distribution of hairy galinsoga is in South and Central America (from Chile to Mexico) and it was introduced to most temperate and subtropical areas of the world (Sweet 1986; Kabuce & Priede 2010; Kosaka *et al.* 2010). Important pathways of its invasion in Europe include escaped individuals from botanical gardens, contamination of soil or seeds of ornamental plants, and transport vehicles. *G. ciliata* is considered as weedy plant naturalized in many European countries (Kabuce & Priede 2010). In its primary and secondary range, it grows particularly in urban areas in gardens, greenhouses, cultivated fields, roadsides, railways and dump sites (Kabuce & Priede 2010), but it was also recorded by Chmura (2004) from semi-natural woodlands.

2.3.2 Seed sources

Seeds of studied species were acquired from several distinct sources by the author if not stated otherwise. Seeds of *S. inaequidens* and *S. viscosus* were collected at the Bubny railway-station in Prague (50°6'5"N, 14°26'25"E) in December 2011. Seeds of *S. jacobaea* were obtained from a commercial company [http://www.plantanaturalis.com]. Seeds of *Galinsoga ciliata* were gathered at Vlásenice (49°27'56"N, 14°33'58"E) in 2009. Nevertheless, their amount was insufficient for further experiments. Therefore, collected seeds of *Galinsoga ciliata* were sown on surface of moist soil consisting of garden substrate (Agro CS a.s.) and sand (1:1) and plants were exposed to 15–30 °C in a greenhouse in spring 2011. Sufficient amount of ripe seeds was then harvested and stored for experiments. Seeds of *Tridax procumbens* produced in different years (1-3 years old) were provided by Prague Botanic Garden.

2.3.3 Preliminary examination of germination rate

The germination rate of studied species was verified in constant conditions before the start of main experiments. Seeds of *Senecio* species (100 seeds per species), *G. ciliata* (50 seeds) and *T. procumbens* (50 seeds) were planted out on moist soil (1 part of garden substrate: 1 part of sand) and kept in 21 °C, 16 hours of light a day. Number of emerged individuals was recorded once per 1–6 days for each species. Examination of germination rate was ended after one month.

2.3.4 Laboratory germination along a temperature gradient

Seed germination response was investigated by exposing seeds of studied species to a temperature gradient (0–32 °C). On 3 May 2012, 25 six-well culture plates were distributed across the gradient in five rows (1-5) and five columns (A-E). Seeds were randomly divided into wells except that there were always same species placed to two 3-well lines in a column (Suppl. Mat. 2). Exact number of seeds (15) was placed into each well on filter paper, watered by 500 μl of tapped water and plates were covered with plastic wrap and lid. Water has been constantly supplied and number of germinated seeds has been counted every 1–3 days for one month. Finally, temperatures in corners of every plate (36 positions) and positions of plates on the gradient table were measured.

2.3.5 Field sowing experiment

Experimental site consisted of 30 1×1 m plots (ten plots with three replications). Fifteen plots were situated within the zone above steam pipeline (zone A) and the rest was set up sixteen meters farther (zone C, close to most remote vegetation plots). Every plot comprised five subplots (25×25 cm in size), from which aboveground and belowground (the upper five centimeters) biomass was removed before sowing. Randomized experimental design was used so that every subplot contained seeds of random species and every plot contained all five species.

On 4 May 2012, seeds were scattered on a topsoil in the centers (10×10 cm) of subplots and moderately watered. Exactly 100 seeds of *Senecio* species and 50 seeds of *Galinsoga* and *Tridax* species were dispersed per subplot. Numbers of emerged seedlings and established plants were recorded once per 6–12 days and subplots were weeded weekly. Sometimes individuals of one species emerged in a plot determined to another species. They were removed when they visibly inhibited original species.

Timing of phenological events such as emergence of first buds or flowers and survival of studied species was observed and recorded in both zones.

2.3.6 Seed viability of *Tridax procumbens*

Mature seeds of *T. probumbens,* collected from zones A and B, were separately counted and 200 seeds from every zone (2×200) were used for further examination. One quarter of seeds (2×50 seeds) was tested for germination rate in a laboratory as described previously (see paragraph *Examination of germination rate*). Three quarters of seeds (2×150 seeds) were sown back to subplots on 27 November 2012. From ten A-subplots, five A-subplots received 15 seeds collected from A-subplots (5×15), and the other five A-subplots received 15 seeds collected from C-subplots (5×15). Ten C-subplots were treated identically as A-subplots.

2.4 Data Analyses

2.4.1 Vegetation data analyses

All analyses were performed and visualized in Canoco 5, Windows release 5.0 (Ter Braak & Šmilauer 2012). Species data were logarithmically transformed [y' = log (1*y + 1)] and the weight of rare species was decreased during every analysis (Lepš & Šmilauer 2003).

2.4.1.1 Multiple sites analyses

All obtained data were tested at once by Detrended Correspondence Analysis (DCA) for any seasonal dynamics of plants occurring in zone A, B, or C. Subsequently, Canonical Correspondence Analysis (CCA) was conducted to evaluate how much data variation was explained by presence or absence of the pipeline. Since the number of plots at the second site was not consistent with other sites, neither permutation tests nor hierarchical design was implemented.

Moreover, the permutation test in CCA could not be initiated with restriction to two sites because there were two zero samples in data. A Distance-based Redundancy Analysis (RDA; Legendre & Anderson 1999) had to be added using Gower distance measure for calculating relevé distances and position of plots (A, B, C) as explanatory variables. Plant species were projected as supplementary data and a hierarchical design with one observation indicating a split-plot and a site representing a whole-plot was set up. A Monte-Carlo permutation test with 499 permutations was executed at a 0.05 level of significance.

2.4.1.2 Single sites analyses

Data from each site were analyzed independently by DCA, CCA and Distance-based RDA. One zero-sample was present in all data sets, so application of Distance-based RDA was inevitable. Constrained ordination diagrams were created by CCA, but significance level was calculated by Distance-based RDA.

2.4.2 Laboratory germination analyses

Coordinates of plates on the gradient table and corresponding measured temperature values were analyzed by a loess model (with span parameter set to 1/3; Fig. 2, Fig. 3) to obtain interpolated temperature values for each well. Relating these temperatures and total proportion of germinated seeds by a Generalized Linear Model (GLM) with quasibinomial distribution provided an estimate of optimum temperatures for species germination. Another GLM with quasibinomial distribution was used to estimate germination rates of the studied species. All analyses were conducted in R, version 2.15 (R Development Core Team, 2012).

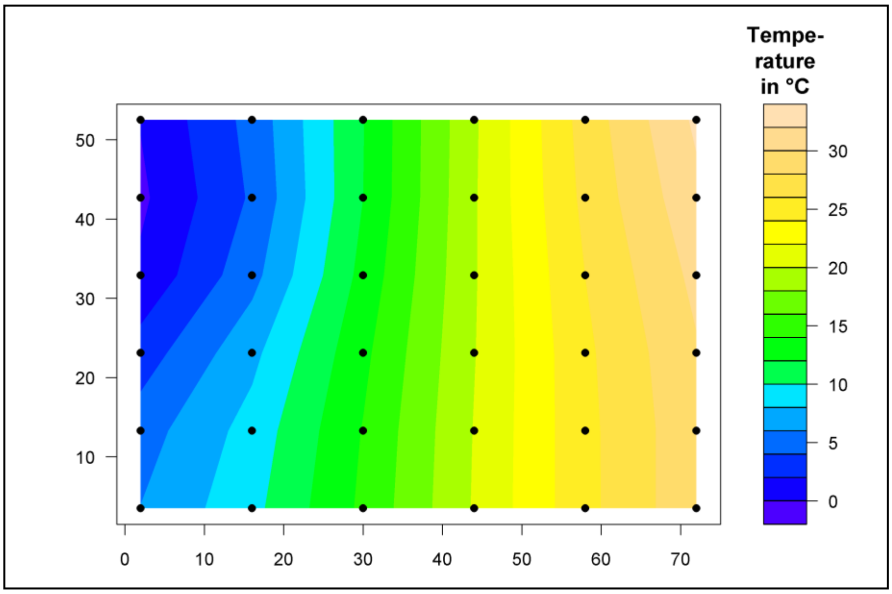

Fig. 2. Temperature gradient fitted by the loess model. Points represent the places, where temperature was measured.

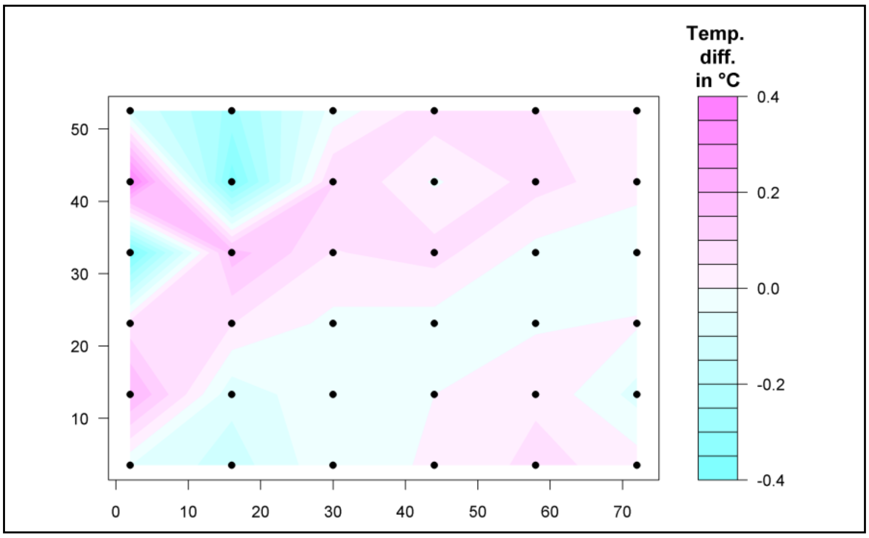

Fig. 3. Temperature differences (°C) between actual measured and model-fitted values. The maximal difference between the loess model fit and actual measured values was 0.4 °C.

2.4.3 Field sowing analyses

Observed numbers of germinated seeds were also analysed by GLM with quasibinomial distribution in R, version 2.15 (R Development Core Team, 2012).

3. Results

3.1 Vegetation data

During the two-year observation of study plots, 69 plant species were recorded in 990 phytosociological relevés. Site 1 contained 46 species and 42 species were found at site 2 and 3. I distinguished 26 non-native species: 20 neophytes and 6 archaeophytes. Raw vegetation data are available at petra.svetlikova@seznam.cz.

3.2 Vegetation data analyses

3.2.1 Multiple sites analyses

Testing all the sites together by Detrended Correspondence Analysis (DCA, Fig. 4) showed more apparent seasonal changes of vegetation in zone A than in zones B and C. Differences between sites suggest separation of the third site indicating dissimilarity between this site and other sites (Fig. 4). The continuous transition from zone A to zone C is noticeable (Fig. 4). First and second canonical axes explained 14.2 and 9.0% of variation.

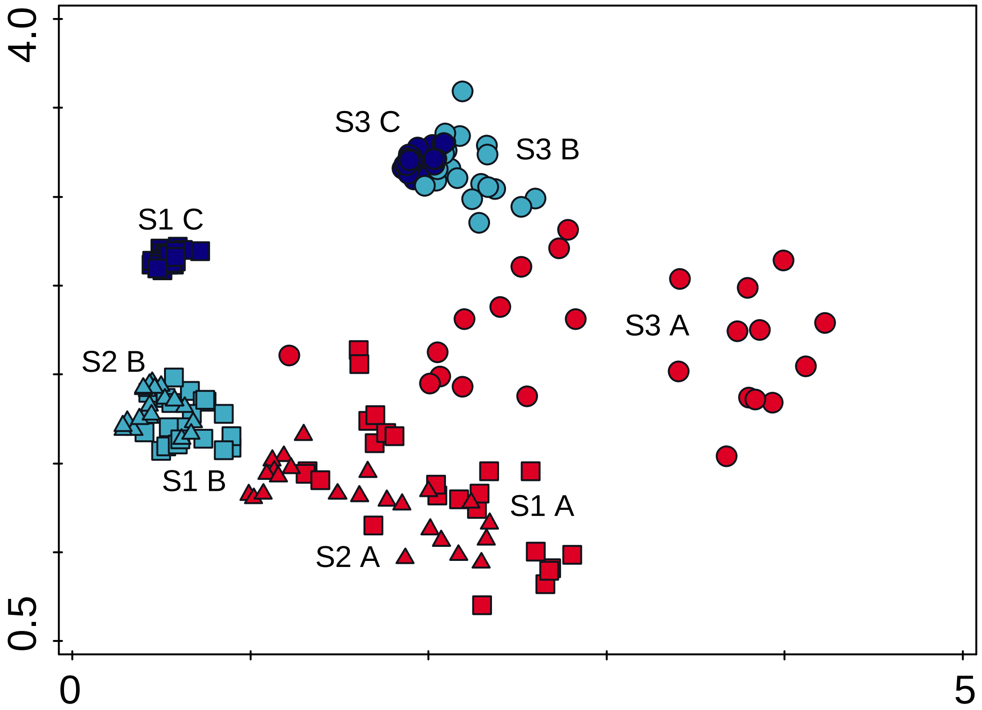

Fig. 4. Unconstrained ordination method (DCA) showing seasonal changes of vegetation at the three study sites (S1-S3) during two years (June 2010 – June 2012). Symbols in red correspond to the pipeline zone (A), -Ssymbols in light-blue to plots 0.5 meters outside the zone (B) and symbols in dark-blue to the most distant plots (C). The first site is presented by squares, the second site by triangles and the third site by circles. Each symbol denotes to the composition of vegetation in five plots during one observation time (centroids used).

The position of observed species and the participation of non-native species especially in the zone A (right bottom) are derived from the same analysis (Fig. 5).

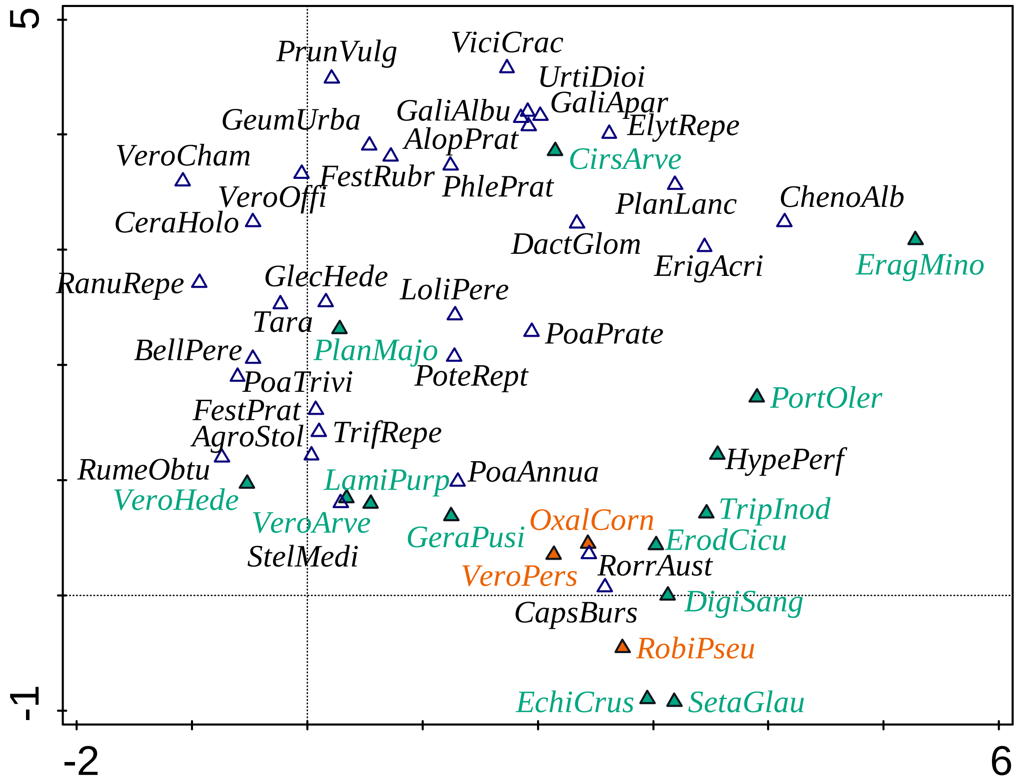

Fig. 5. Unconstrained ordination method (DCA) displaying positions of 50 plant species with largest weight. Non-native species are highlighted, neophytes by orange colour and archaeophytes by green colour. Abbreviations are explained in Supplementary Material 3.

Canonical Correspondence Analysis (CCA) covering all three sites (Fig. 6) or only sites 1 and 2 (Fig. 7) implies usual habitat of species with regard to zones A, B and C. First and second canonical axes accounted for 6.3 and 3.8% of variation of all-sites dataset, while first and second canonical axes explained 9.3 and 4.1% of variation of two-site dataset. Zones are equally distributed from each other (Fig. 6, 7). Although zones A and B have many species in common, almost no species occur in zone A and C at the same time (Fig 6, 7).

Distance-based RDA diagrams inferred from all three datasets or from first and third datasets are not shown, but both axes in both analyses explained more variability than in CCA. First and second canonical axes accounted for 7.3 and 6.3% of variation of all-sites dataset, while first and second canonical axes explained 9.9 and 6.1% of variation of two-site dataset. Permutation test with three explanatory variables (A, B, C) conducted only for data from site 1 and 3 is significant (P = 0.002) suggesting that vegetation of these three zones is significantly different.

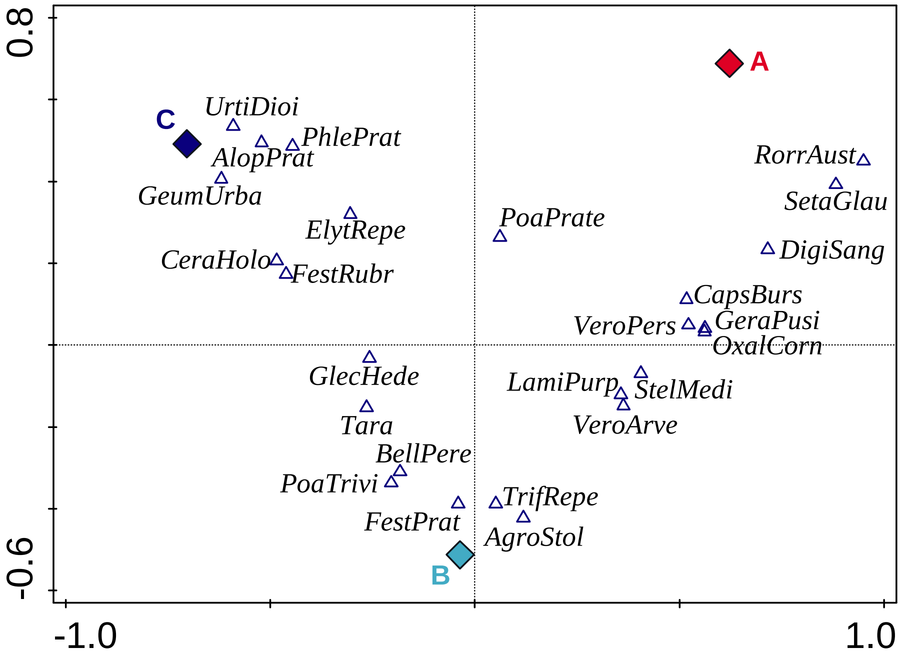

Fig. 6. Constrained ordination method (CCA) including all data and indicating preferences of 25 best fitted plant species for the pipeline zone (A) or for the outside the zone (B, C). Abbreviations are explained in Supplementary Material 3.

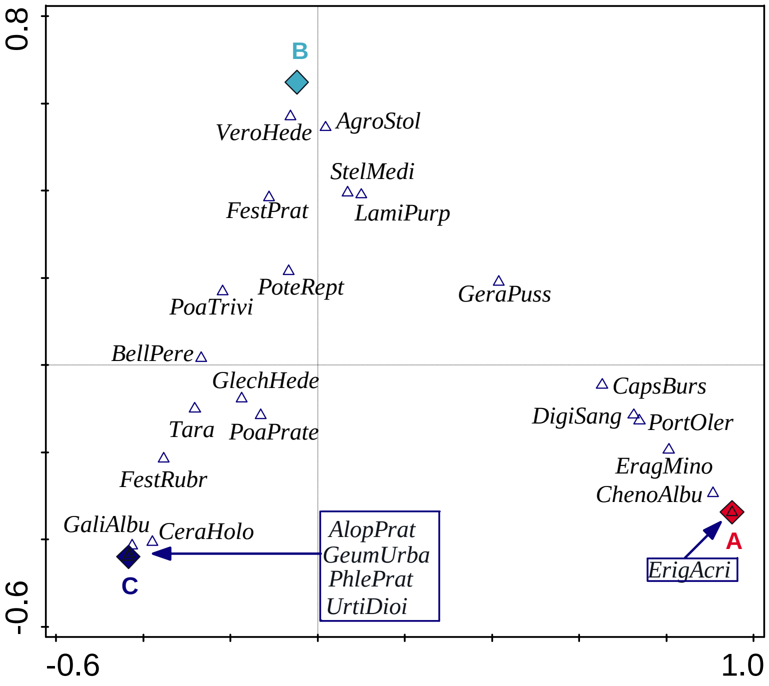

Fig. 7. Constrained ordination method (CCA) including only the first and the third sites and indicating preferences of 25 best fitting plant species for the pipeline zone (A) or for the outside the zone (B, C). Abbreviations are explained in Supplementary Material 3.

3.2.2 Single sites analyses

Site 1

Detrended Correspondence Analysis (DCA) presents seasonal dynamics of vegetation at the first site and demonstrates decreasing amount of changes in vegetation during a year from the zone A to the zone C (Fig. 8). First and second canonical axes explained 20.9 and 9.6% of variation.

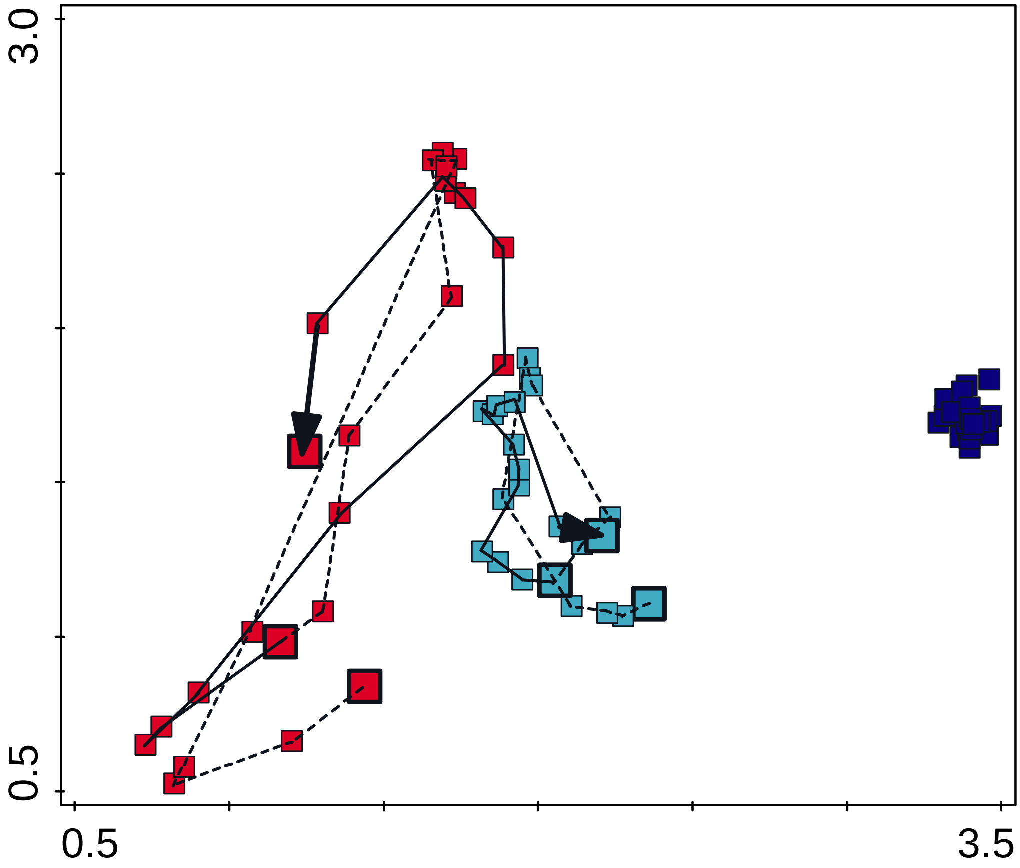

Fig. 8. Seasonal dynamics of vegetation at the first site during two years obtained by unconstrained ordination method (DCA). Symbols in red correspond to the pipelines zone (A), symbols in light-blue to plots 0.5 meters outside the zone (B) and symbols in dark-blue to the most distant plots (C). Every symbol (centroid) denotes to the composition of vegetation in five plots during one observation time. Dashed line indicates first year and solid line indicates second year. Enlarged symbols represent first (June 2010), intermediate (June 2011), and last (June 2012) observations. Arrows show last observations.

Preferences of species for zone A, B or C are shown on constrained unimodal ordination (CCA) diagram (Fig. 9). While within site 1, *Portulaca oleracea* and *Digitaria sanguinalis* growed predominantly in the zone A, *Dactylis glomerata*, *Veronica hederifolia*, and *Agrostis stolonifera* prefered places near the zone A, and *Lolium perenne*, *Taraxacum* sp., and *Cerastium holosteoides* occured farther from it (Fig. 9). First and second canonical axes explained 16.9 and 10.7% of variation.

From Distance-based RDA, first and second canonical axes accounted for 25.2 and 14.5% of variation. Species composition of individual zones is significantly different (P = 0.002).

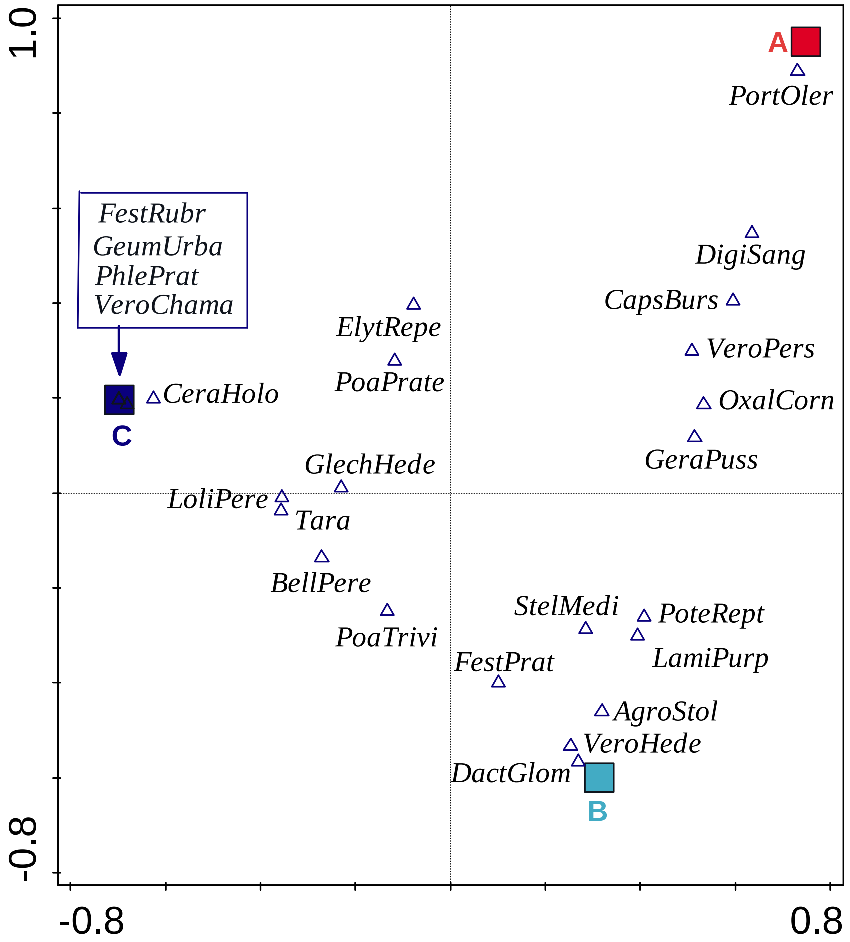

Fig. 9. Constrained unimodal ordination diagram (CCA) representing relationships of 25 best fitted plant species to the pipeline zone (A) or the outside zones (B, C). Abbreviations are explained in Supplementary Material 3.

Site 2

Vegetation at the second site was changing quite rapidly in the zone A when compared to the zone B (Fig. 10). First and second canonical axes of DCA accounted for 22.7 and 9.9% of variation.

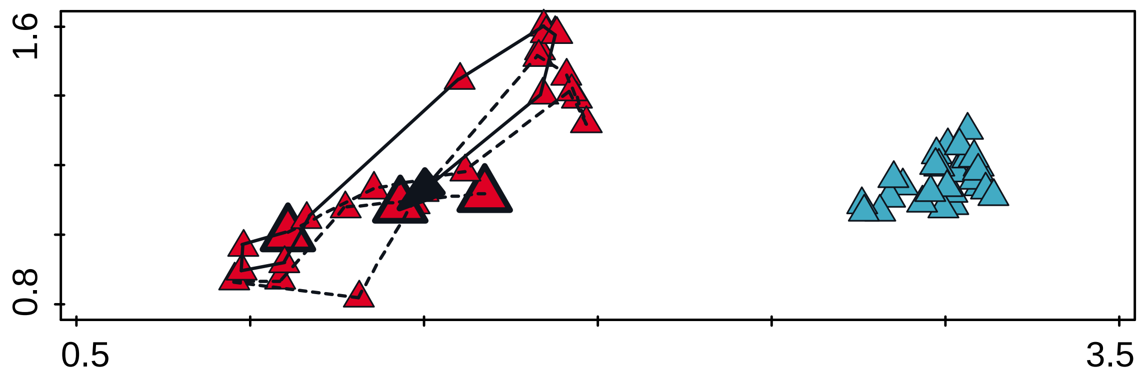

Fig. 10. Seasonal dynamics of vegetation at the second site during two years obtained by unconstrained ordination method (DCA). Symbols in red correspond to the pipelines zone (A) and symbols in light-blue to plots 0.5 meters outside the zone (B). Every symbol (centroid) denotes to the composition of vegetation in five plots during one observation time. Dashed line indicates first year and the solid line indicates second year. Enlarged symbols represent first (June 2010), intermediate (June 2011), and last (June 2012) observations. Arrow shows last observation.

Canonical Correspondence Analysis (CCA) of data from the second site shows preferences of *Rorripa austriaca*, and *Geranium pussilum* for the zone A and preferences of *Poa trivialis*, *Glechoma hederacea* and *Plantago major* for the zone B (Fig. 11). First canonical axis accounted for 18.1% of variation.

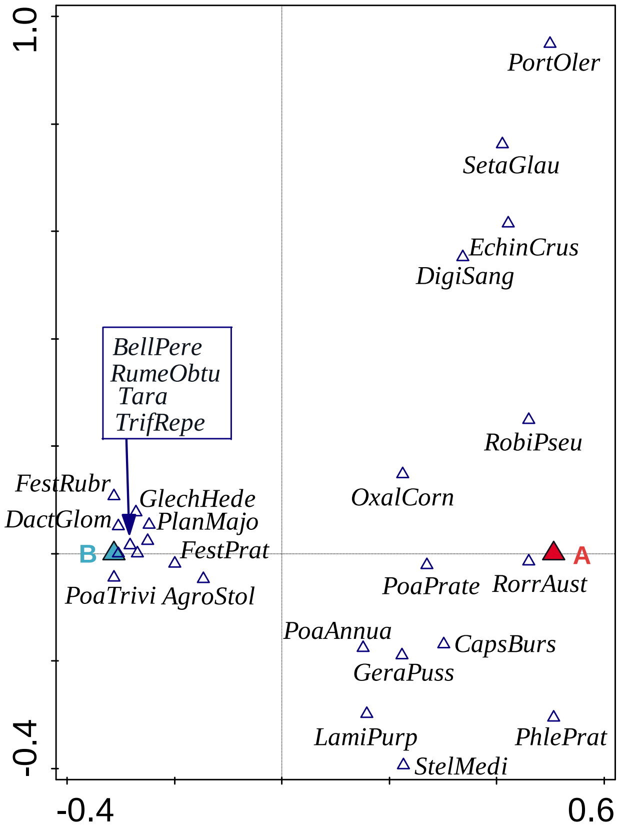

Fig. 11. Constrained unimodal ordination diagram (CCA) representing relationships of 25 best fitted plant species to the pipeline zone (A) or the outside zone (B) at the second site. Abbreviations are explained in Supplementary Material 3.

Same explanatory variables used in Distance-based RDA with hierarchical design explained 24.64% of variation and the vegetation of the zone A is significantly different from the zone B (P = 0.01).

Site 3

Already described trend of greater changes of vegetation in the zone A was also evident at the third site (Fig. 12). First and second canonical axes within the DCA explained 22.4 and 10.9% of variation.

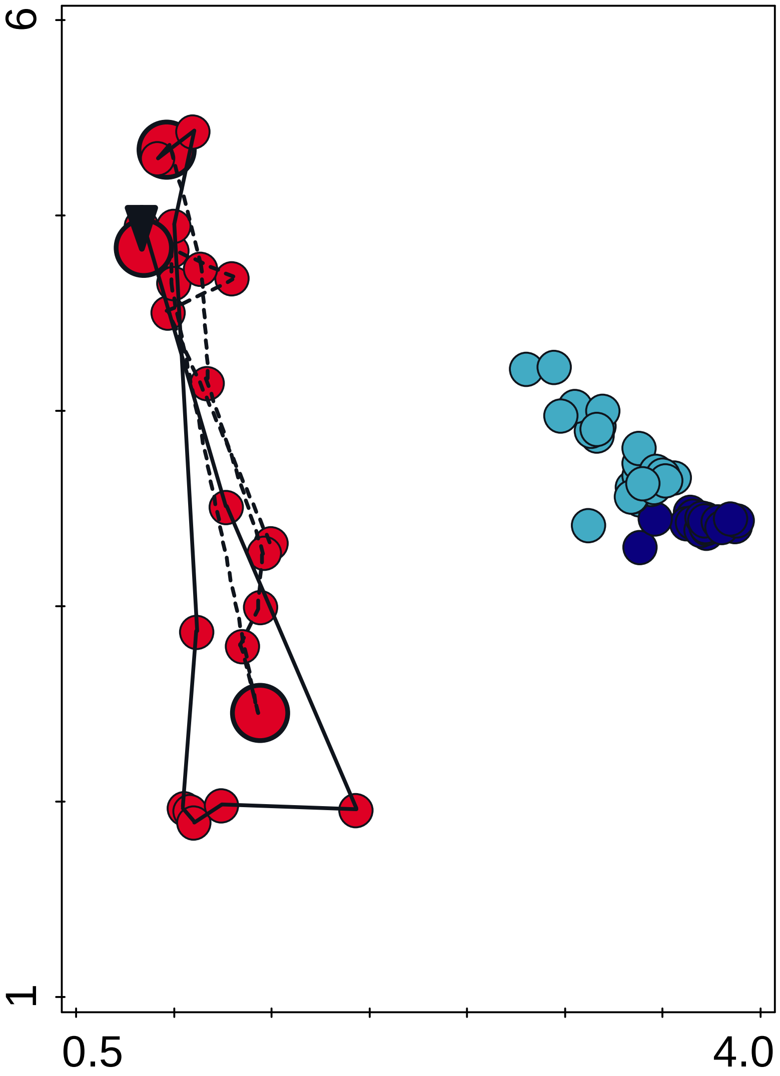

Fig. 12. Seasonal dynamics of vegetation at the third site during two years obtained by unconstrained ordination method (DCA). Symbols in red correspond to the pipelines zone (A), symbols in light-blue to plots 0.5 meters outside the zone (B) and symbols in dark-blue to the most distant plots (C). Every symbol (centroid) denotes to the composition of vegetation in five plots during one observation time. Dashed line indicates first year and the solid line indicates second year. Enlarged symbols represent first (June 2010), intermediate (June 2011), and last (June 2012) observations. Arrow show last observation.

According to the CCA of dataset from the third site, *Tripleurospermum inodorum*, *Erigeron acris*, *Plantago lanceolata*, and *Digitaria sanguinalis* can be considered as typical species for the zone A, whereas *Galium album*, *Aloperurus pratensis*, *Cirsium arvense*, and *Phleum pratense* occured predominantly in the zone B (Fig. 13). Many species were common for zones B and C (Fig. 13). First and second canonical axes accounted for 19.9 and 3.9% of variation.

Although Distance-based RDA explained much more variability than CCA (31.1% vs. 23.7%), most of variability was explained by first canonical axis (31.3% for first as opposed to 1.8% for second axis) suggesting more dissimilarity between zones A and B than between B and C. The composition of vegetation significantly differed between these three zones (P = 0,002).

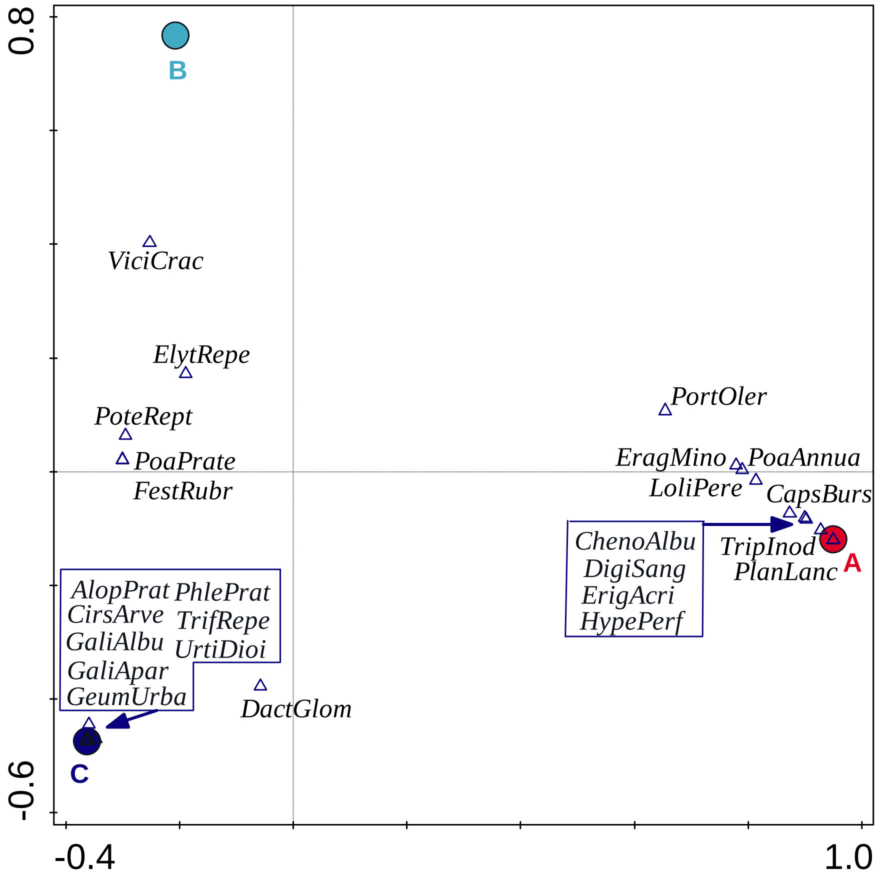

Fig. 13. Constrained unimodal ordination diagram (CCA) representing relationships between 25 best fitted plant species to the pipeline zone (A) or the outside zones (B, C) at the third site. Abbreviations are explained in Supplementary Material 3.

3.3 Soil temperature measurement

Soil temperature strongly decreased with the distance from the pipeline (Fig. 14, 15) suggesting great heating dissipation from the pipeline. A two-year average is more than 13 °C higher above the pipeline than 16 m outside the pipeline (Table 1).

Table 1. Mean soil temperatures (°C) measured in three transects at the study sites for year 1 (August 2010-July 2011) and year 2 (August 2011-July 2012) and comparison with air temperatures. **A** represents the pipeline zone, **A-B** the transition zone, **B** the zone 0.5 m outside the pipeline and **C** the zone 16 m outside the pipeline.

zone	**A**			**A-B**			**B**			**C**			**air**
site	1	2	3	1	2	3	1	2	3	1	2	3	
year 1	22.4	21.9	24.6	21.6	20.1	23.4	17.4	16.4	19.8	10.8	10.8	8.7	8.7
year 2	24.3	23.3	24.5	23.0	21.8	22.8	18.6	17.5	20.3	10.8	12.0	9.4	9.4
two year average	23.5			22.1			18.3			10.3			9.1

Mean air temperatures were found to be very similar to mean temperatures from zone C providing an evidence that these plots are not influenced by heating dissipation off the pipeline. However, zone B was found to be strongly influenced (Fig. 14, 15). Heat from the pipeline thus likely penetrates more than 0.5 meters from the transition zone making a temperature gradient, but no temperature data were sampled for this zone.

Soil temperature maximum was recorded at the third site on 29 June 2011 at 14:30 and reached 53.6 °C (Table 2). Maximum temperatures measured within other sites were much lower, about 40 °C (Table 2). August 2011 was the hottest month at the first and the third site, while July 2012 was the hottest month at the second site.

Table 2. Maximum and minimum soil temperatures (°C) measured at the study sites from July 2010 to July 2012. **A** represents the pipeline zone, **A-B** the transition zone, **B** the zone 0.5 m outside the pipeline and **C** the zone 16 m outside the pipeline.

zone	**A**			**A-B**			**B**			**C**		
site	1	2	3	1	2	3	1	2	3	1	2	3
temperature maximum	40.4	39.7	53.6	37.4	38.6	43.7	30.4	30.6	34.0	29.8	31.2	25.3
temperature minimum	1.7	4.1	3	5.9	4.4	4.7	2.2	0.7	4.2	-5.3	-7.0	-6.4

The second site seems to be colder than other sites (Fig. 14, 15). The steepest temperature gradient was recorded between zones A and C at the third site (Fig. 14, 15). Although the temperature detected in zones C is much lower than in zones A (Table 1, 2), daily courses of temperature follow similar trends regardless the place (Fig. 16, 17).

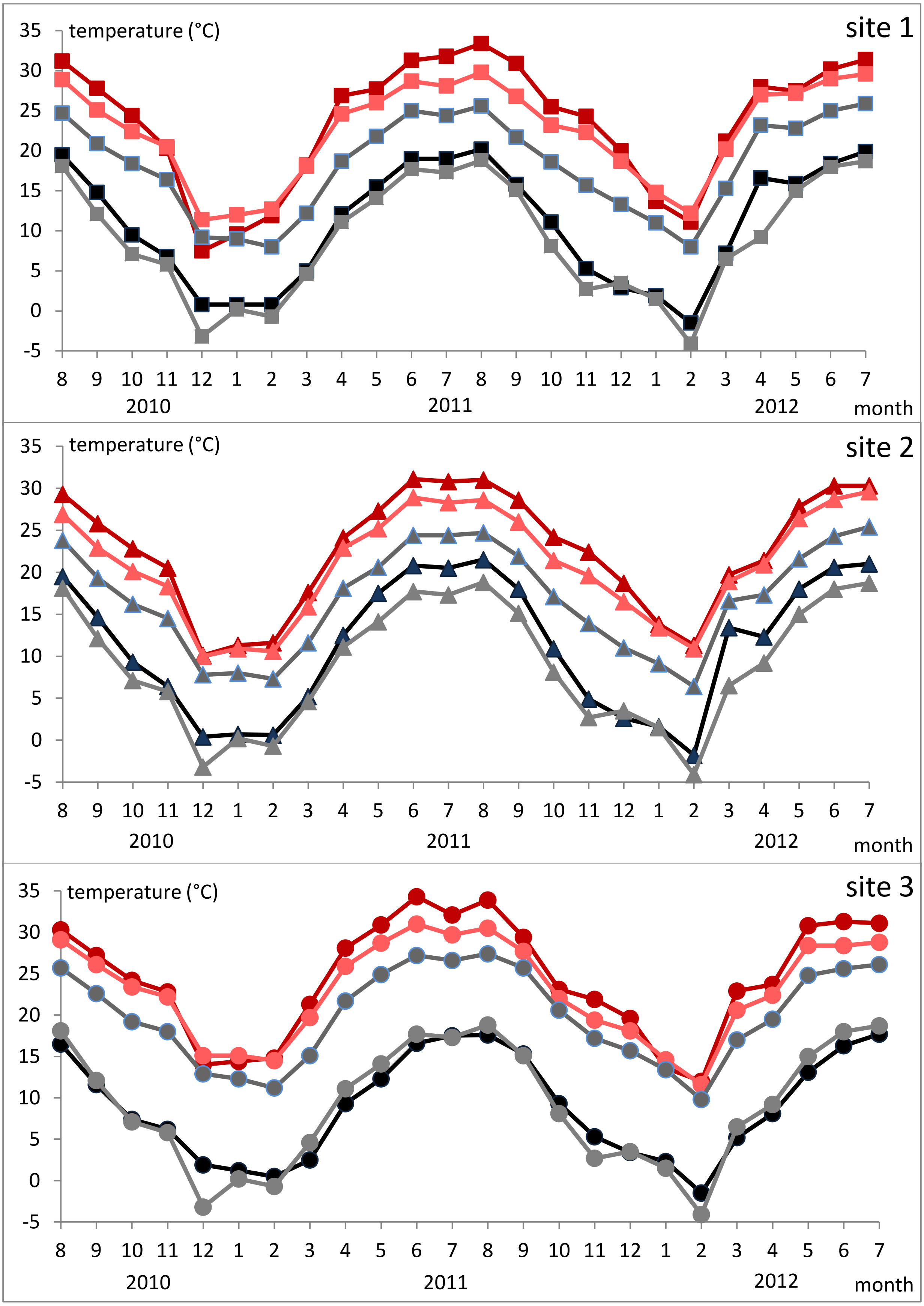

Fig. 14. Dynamics of soil and air temperature (°C) at the study sites during two years. Monthly averages are presented. Curves in red indicate the middle of the pipeline zone (zone A), curves in pink indicate the transition zone (zone A-B, the margin of the pipeline zone), curves in blue the zone 0.5 m from the pipeline (zone B), curves in dark blue the zone 15 m from the previous (zone C), and curves in gray air temperature. Air temperature data were provided by CHMI (Czech Hydrometerological Institute) in České Budějovice.

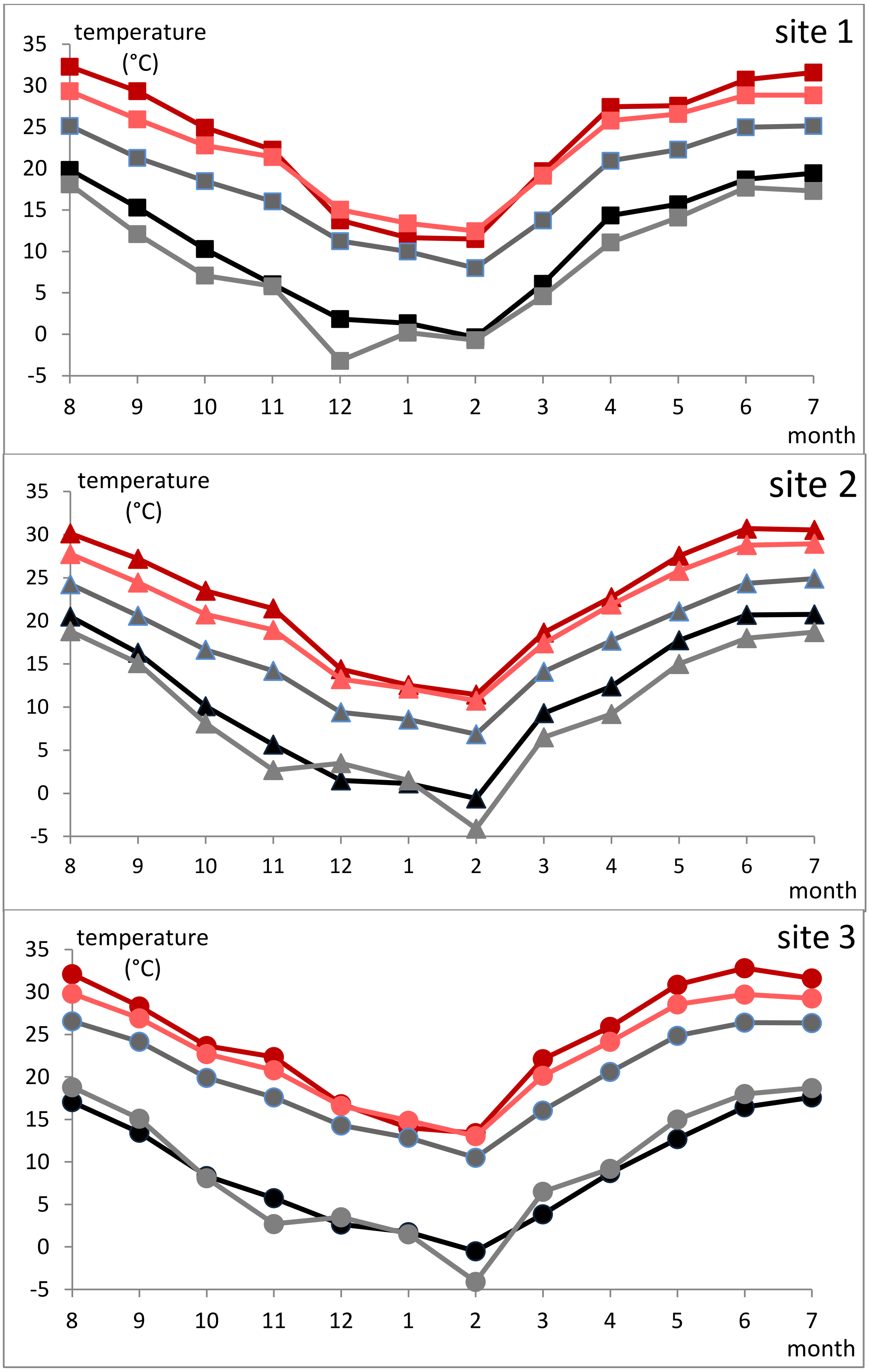

Fig. 15. Averaged monthly temperatures (°C) calculated from two-year data measured at the study sites. Curves in red represent the middle of the pipeline zone (zone A), curves in pink the transition zone (zone A-B, the margin of the pipeline zone), curves in blue the zone 0.5 m from the pipeline (zone B), curves in dark blue the zone 15 m from the previous (zone C), and gray represent air temperature.

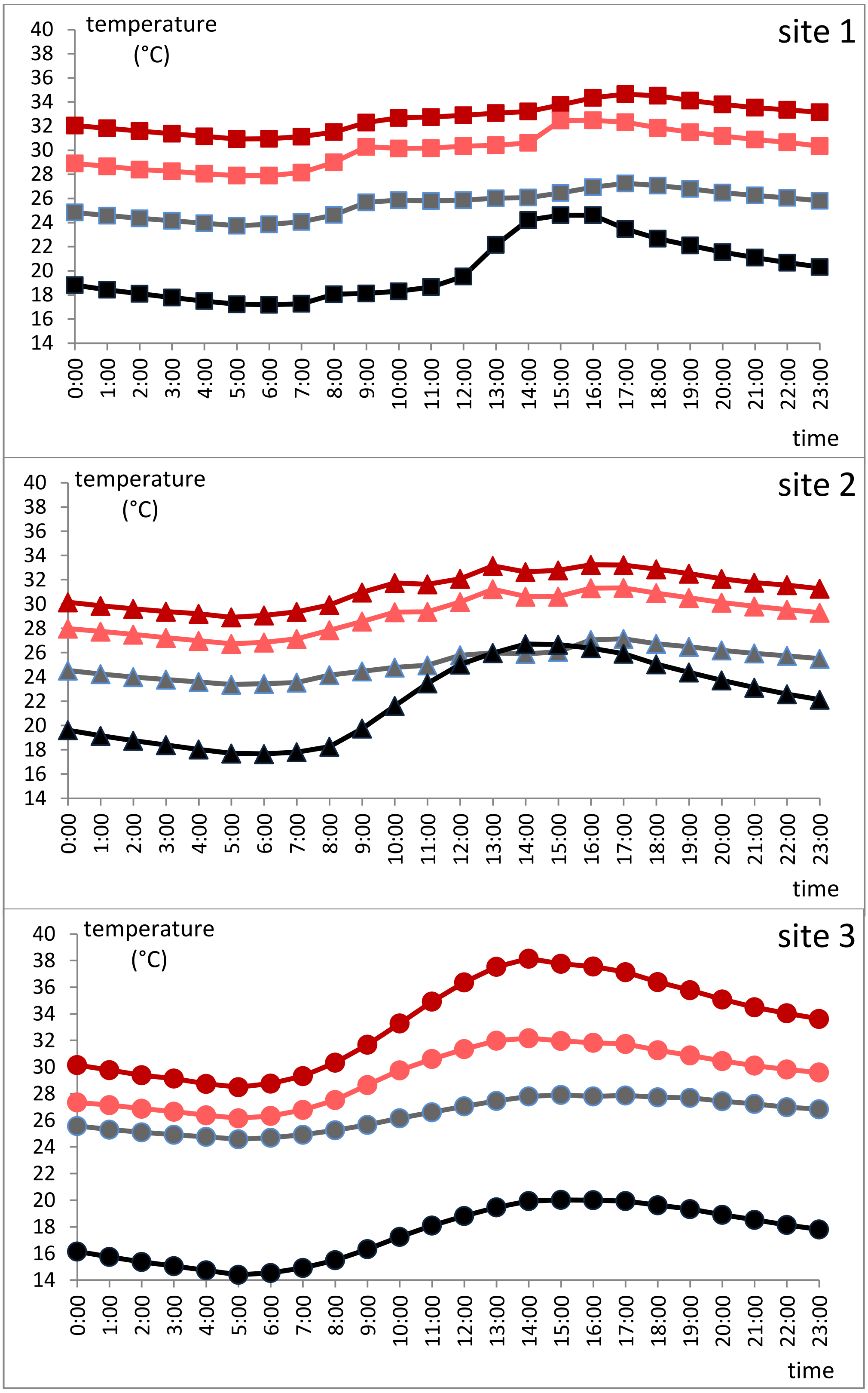

Fig. 16. Daily course of soil temperature (°C) at the study sites on sunny summer day (3 August 2011). Curves in red correspond to the middle of the pipeline zone (zone A), curves in pink to the transition zone (zone A-B, the margin of the pipeline zone), curves in blue to the zone 0.5 m from the pipeline (zone B) and curves in dark blue to the zone 15 m from the previous (zone C).

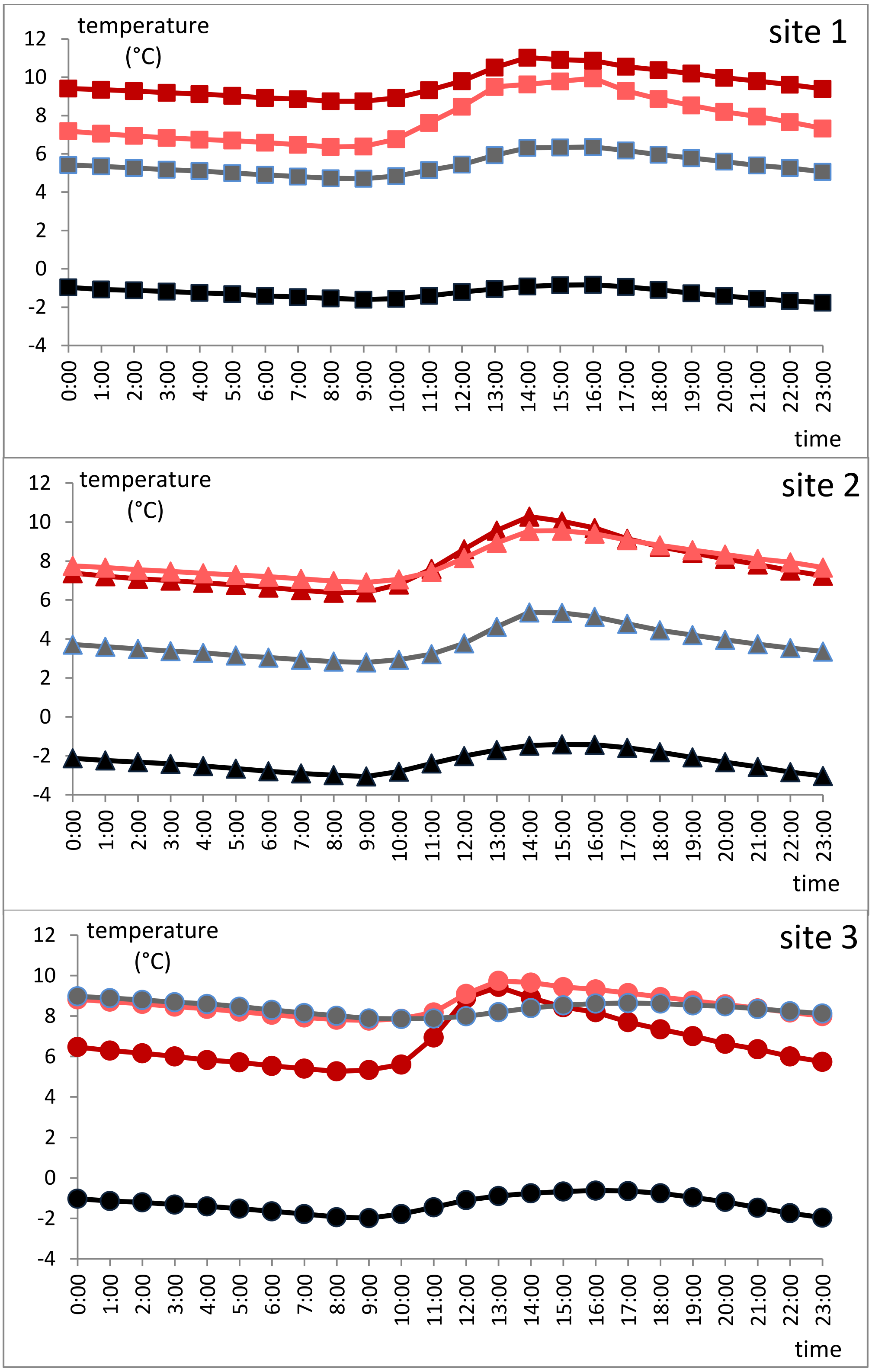

Fig. 17. Daily course of soil temperature (°C) at the study sites on sunny winter day (1 February 2012). Curves in red correspond to the middle of the pipeline zone (zone A), curves in pink to the transition zone (zone A-B, the margin of the pipeline zone), curves in blue to the zone 0.5 m from the pipeline (zone B) and curves in dark blue to the zone 15 m from the previous (zone C).

3.4 Soil moisture measurement

Soil moisture gradually increased with the distance from the pipeline (Fig. 18) indicating strong water vapor above the pipeline. Average volumetric water content measured from 1 December 2012 for a year was about 15% lower in the zone A compared to the zone B. Data from zones A-B and C are available only from December 2011 to February 2012, because dataloggers were broken.

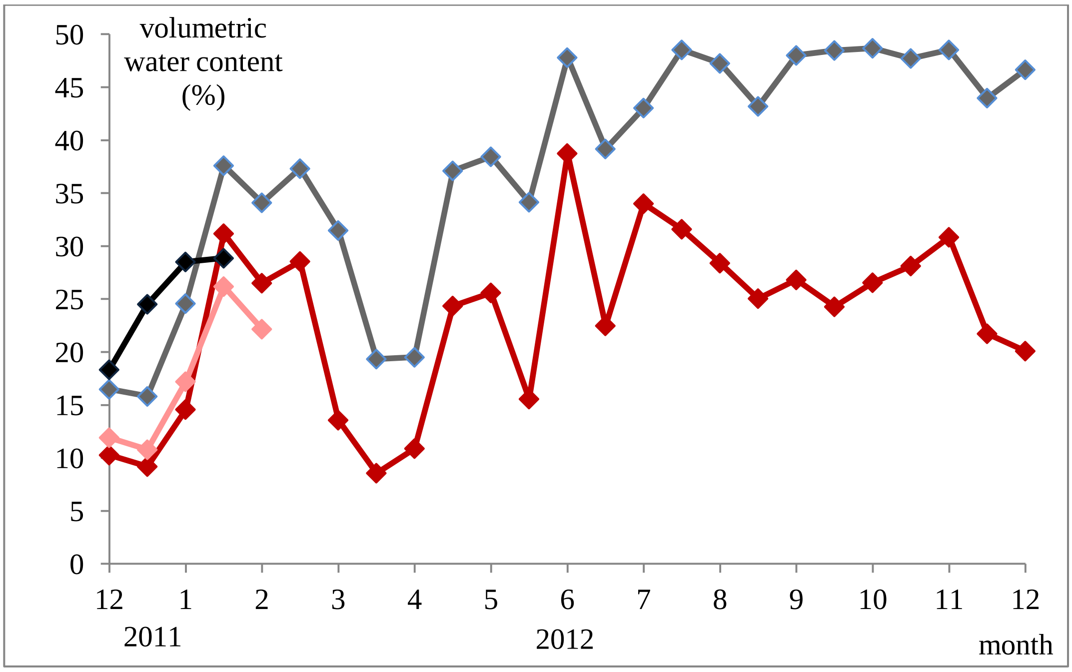

Fig. 18. Course of soil moisture expressed as volumetric water content (%) at the second study site during one year. Two-week averages are presented. Curves in red indicate the middle of the pipeline zone (zone A), curves in pink indicate the transition zone (zone A-B, the margin of the pipeline zone), curves in blue the zone 0.5 m from the pipeline (zone B), curves in dark blue the zone 15 m from the previous (zone C), and curves in gray air temperature.

3.4 Sowing experiments

3.4.1 Preliminary examination of germination rate

Total germination rate of studied species tested under fixed conditions after a month was 88% for *G. ciliata*, 62% for *S. inaequidens*, 48% for *S. jacobaea*, 35% for *S. viscosus,* and 0% for *T. procumbens*. Most seeds germinated within first two weeks (Table 3).

Table 3. Numbers of germinated seeds recorded every 1-6 days for a month. First column indicates number of days from sowing (day 0) and numbers in brackets correspond to the number of sown seeds.

species **day**	*Galinsoga ciliata* (50)	*Senecio inaequidens* (100)	*Senecio jacobaea* (100)	*Senecio viscosus* (100)	*Tridax procumbens* (50)
7	0	0	3	0	0
9	12	1	23	5	0
10	17	15	28	5	0
11	27	35	32	6	0
13	40	50	41	18	0
14	40	52	43	23	0
18	43	65	48	31	0
19	44	65	48	32	0
25	44	55	48	35	0
27	44	62	47	31	0

3.4.2 Laboratory germination experiment

Exposing seeds to the temperature gradient revealed optimal germination temperatures of studied species (Fig. 19). For *S. jacobaea* and *S. inaequidens* optimal germination temperature was at approximately 18 °C, whereas optimal germination temperatures for remaining species were higher. *G. ciliata* showed highest proportion of germination at 22-28 °C and *S. viscosus* and *T. probumbens* germinated predominantly at about 30 °C (Fig. 19). Proportion of germinated seeds significantly differed between species and across temperature gradient (Table 4).

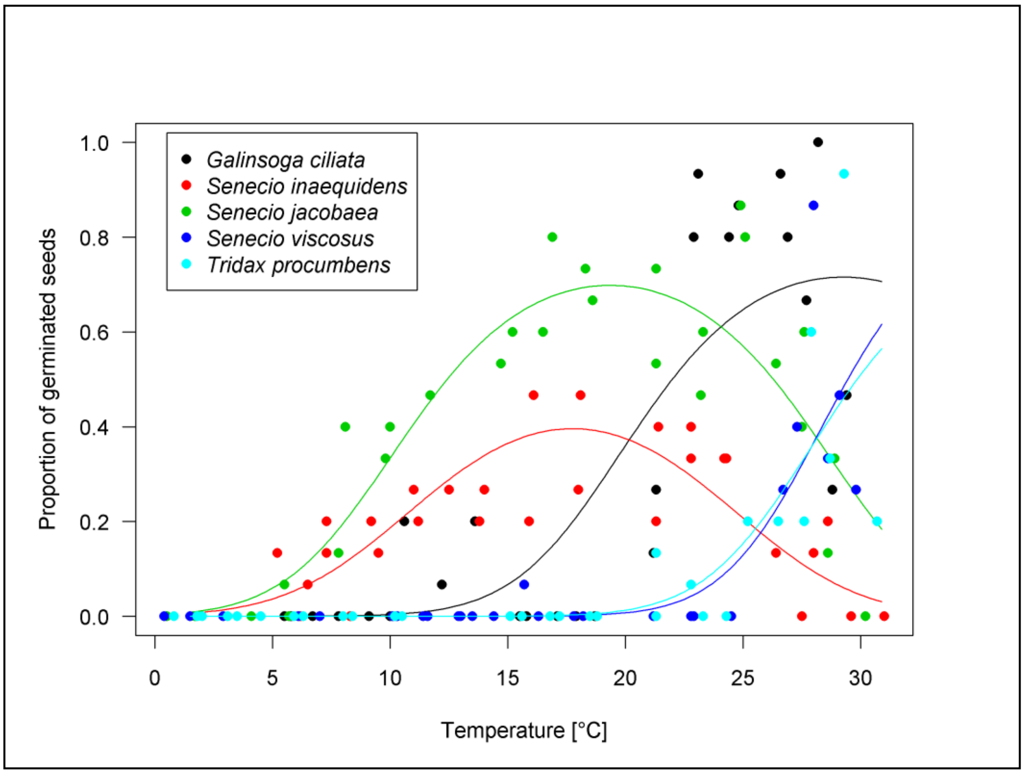

Fig. 19. Proportion of germinated seeds of studied species under the temperature gradient after one month. Only *S. jacobaea*, *S. inaquidens*, and *G. ciliata* are accurately fitted by the model.

Table 4. Summary of the quasibinomial generalized linear model (GLM) testing the effect of species, temperature and interaction of species and temperature on proportion of germinated seeds of studied species. Statistically significant test results (P <0.05) are highlighted in bold.

Effect	df	Deviance	Residual deviance	F	P
Species	4,145	11.95	60.38	20.43	**$< 10^{-12}$**
Temperature (linear trend)	1,144	20.50	39.88	140.26	**$< 10^{-15}$**
Temperature^2 (2nd –degree polynomial trend)	1,143	2.17	37.71	14.83	**< 0.001**
Species × temperature	4,139	20.16	17.55	34.48	**$< 10^{-15}$**
Species × temperature^2 (2nd –degree polynomial trend)	4,135	0.37	17.18	0.63	0.6434

Proportion of germinated seeds within a half time of the experiment and the final number of germinated seeds showed the germination rate of studied species (Fig. 20). The fastest germination was recorded for *S. jacobaea*, followed by *S. inaequidens*, *T. procumbens*, *G. ciliata* and *S. viscosus* (Fig. 20). Decreasing curves imply an acceleration of germination time by higher temperatures for all the species.

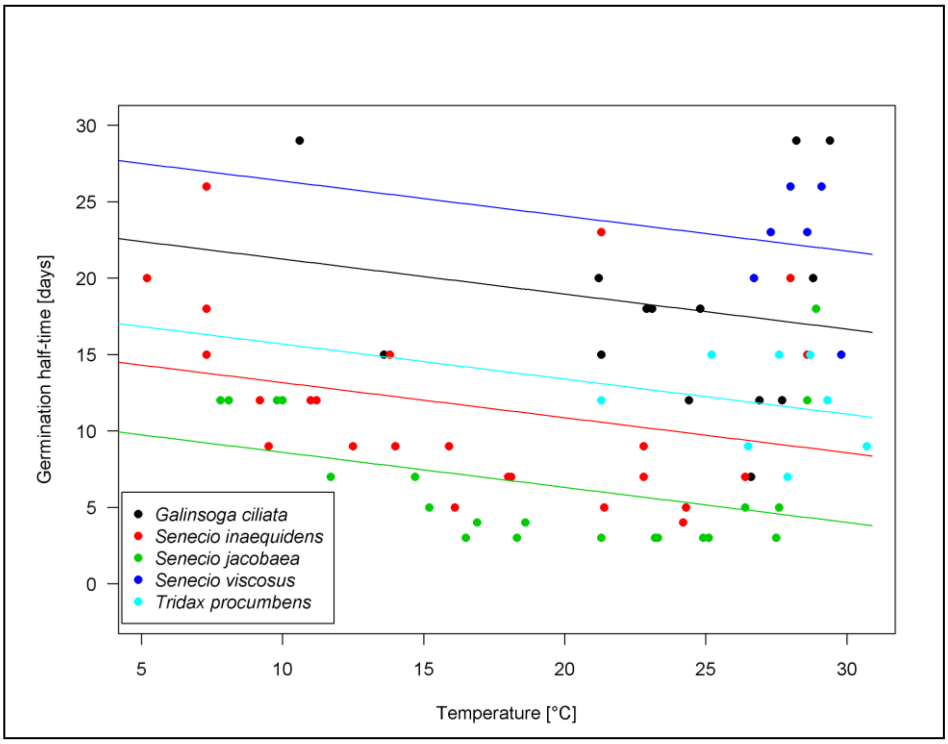

Fig. 20. Germination rate of studied species under the temperature gradient. Germination half-time relates to the number of days when more than half of total germinated seeds reached germination. Only *S. jacobaea*, *S. inaquidens*, and *G. ciliata* are accurately fitted by the model.

3.4.3 Field sowing experiment

Course of germination observed during the first 57 days after sowing was not different between zones A and C for any studied species except *T. procumbens* (Fig. 21-25, Table 5). *T. procumbens* showed a higher proportion of germinated seeds in the zone A in every observation time (Fig. 22). However, overall proportion of germinated seeds of *T. procumbens*, *S. viscosus* and *S. jacobaea* was very small regardless a zone (Fig. 22, 24, 25). *S. viscosus* was found to germinate preferentialy in the zone C (Fig. 25).

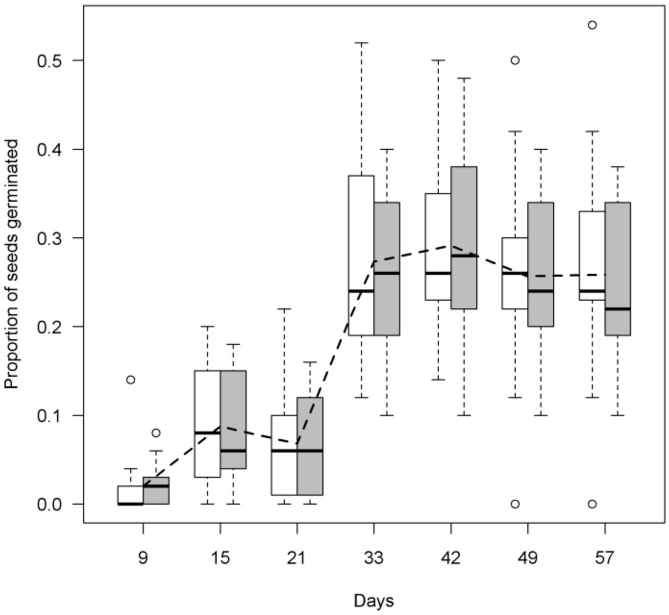

Fig. 21. Course of germination of *Galinsoga ciliata* above the pipeline (zone A, gray boxes) and outside the pipeline (zone C, white boxes) during 57 days after sowing. Boxes indicate 25% and 75% quantiles and whiskers indicate nonoutlier range of values. Empty circles represent outliers. Proportion of germinated seeds within observed days was not significantly different between zones A and C (P = 0.6134, Table 5).

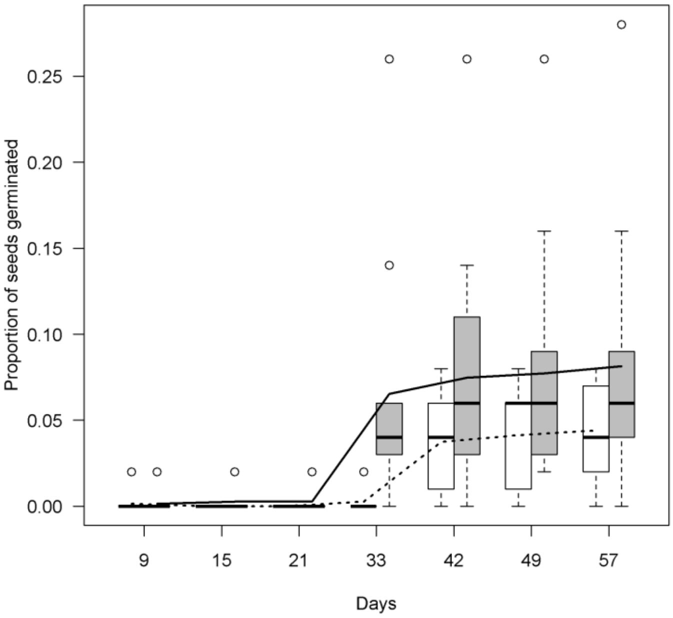

Fig. 22. Course of germination of *Tridax procumbens* above the pipeline (zone A, gray boxes, solid line) and outside the pipeline (zone C, white boxes, dotted line) during 57 days after sowing. Boxes indicate 25% and 75% quantiles and whiskers indicate nonoutlier range of values. Empty circles represent outliers. Numbers of germinated seeds significantly differed between zones A and C ($P < 10^{-8}$, Table 5).

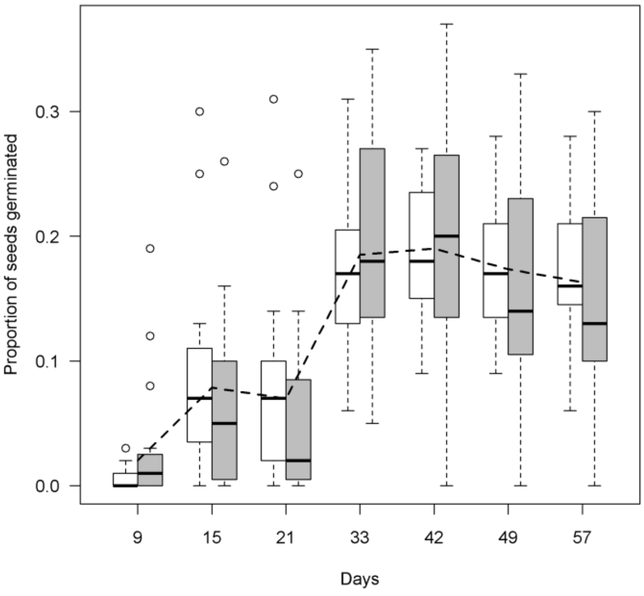

Fig. 23. Course of germination of *Senecio inaequidens* above the pipeline (zone A, gray boxes) and outside the pipeline (zone C, white boxes) during 57 days after sowing. Boxes indicate 25% and 75% quantiles and whiskers indicate nonoutlier range of values. Empty circles represent outliers. Numbers of germinated seeds were not significantly different between zones A and C (P = 0.8756, Table 5).

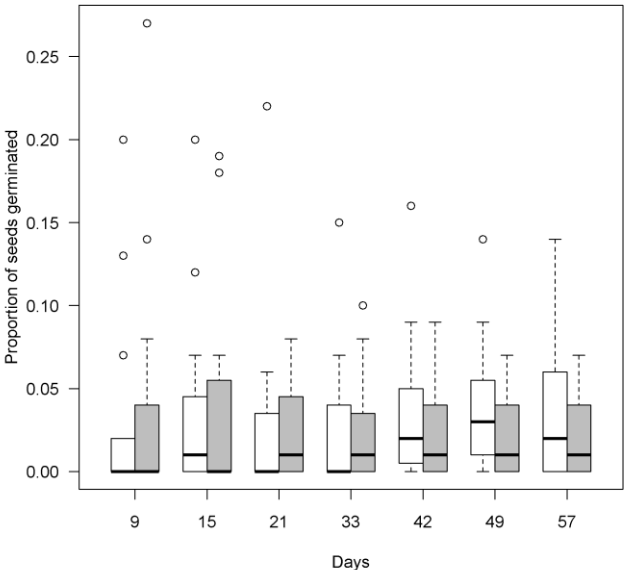

Fig. 24. Course of germination of *Senecio jacobaea* above the pipeline (zone A, gray boxes) and outside the pipeline (zone C, white boxes) during 57 days after sowing. Boxes indicate 25% and 75% quantiles and whiskers indicate nonoutlier range of values. Empty circles represent outliers. Numbers of germinated seeds were not significantly different between zones A and C (P = 0.4565, Table 5).

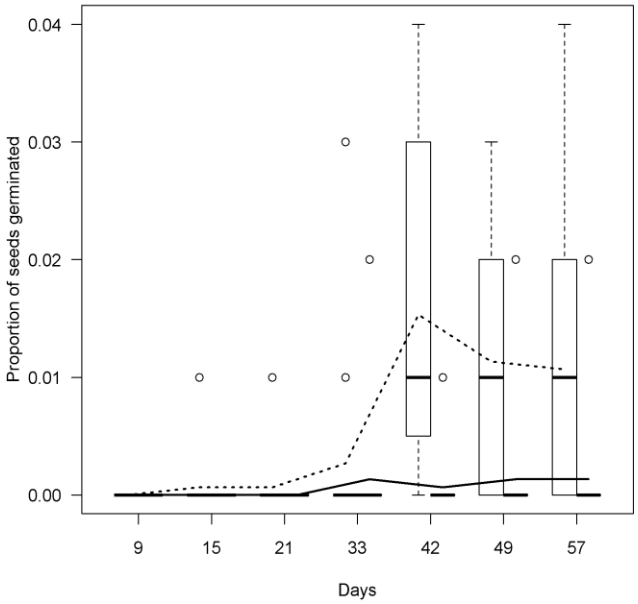

Fig. 25. Course of germination of *Senecio viscosus* above the pipeline (zone A, solid line) and outside the pipeline (zone C, white boxes, dotted line) during 57 days after sowing. Almost no seeds germinated above the pipeline. Boxes indicate 25% and 75% quantiles and whiskers indicate nonoutlier range of values. Empty circles represent outliers. Numbers of germinated seeds were not significantly different between zones A and C (P = 0.8756, Table 5).

Table 5. Summary of the general linear model (GLM) testing the effect of day, pipeline, and interaction of day and pipeline on course germination of studied species. GC indicates *G. ciliata*, TP *Tridax procumbens*, SI *Senecio inaequidens*, SJ *S. jacobaea*, and SV *S. viscosus*. Statistically significant test results ($P < 0.05$) are highlighted in bold.

Species	Effect	df	Deviance	Residual deviance	F	P
GC	day	6, 203	19.13	12.67	53.43	**$<10^{-15}$**
	pipeline	1, 202	0.02	12.65	0.26	0.6134
	day × pipeline	6, 196	0.03	12.63	0.08	0.9982
TP	day	6, 203	6.59	7.02	35.77	**$<10^{-15}$**
	pipeline	1, 202	1.21	5.81	39.26	**$<10^{-8}$**
	day × pipeline	6, 196	0.57	5.24	3.09	**0.0064**
SI	day	6, 203	8.96	12.12	25.2	**$<10^{-15}$**
	pipeline	1, 202	0	12.12	0.02	0.8756
	day × pipeline	6, 196	0.49	11.63	1.39	0.2223
SJ	day	6, 203	0.11	11.95	0.28	0.9481
	pipeline	1, 202	0.04	11.91	0.56	0.4565
	day × pipeline	6, 196	0.17	11.75	0.4	0.8798
SV	day	6, 203	8.96	12.12	25.2	**$<10^{-15}$**
	pipeline	1, 202	0	12.12	0.02	0.8756
	day × pipeline	6, 196	0.49	11.63	1.39	0.2223

First flowers of *G. ciliata* were recorded on 20 July. At the same time, I recorded first buds of *T. procumbens* which converted to flowers in a week. Buds were observed earlier in the zone A than in B, estimate difference is about 7 days. Remaining species except *S. jacobaea* flowered later, *S. inaequidens* flowered from September to December, *S. viscosus* in early September. Unfortunately, phenology of flowered *Senecio* species was not comparable between zone A and C, because both zones were not cut at the same time from the end of summer.

Although *Senecio* species survived longer than the remaining species, all species except *S.viscosus* (grew only in zone C) survived longer in the zone A. *G. ciliata* and *T. procumbens* were observed about 30 days longer in the zone A than in C. *S. jacobaea* is still (12 December) surviving in the zone A and *S. inaequidens* is surviving in both zones.

3.4.4 Seed viability of *Tridax procumbens*

Seed viability test is still in progress, first seedlings are starting to emerge from seeds collected in zones A and C.

4. Discussion

4.1 Vegetation above the pipeline

Vegetation above the pipeline is characterized predominantly by short-lived plant species. While common native species dominate above the pipeline from the end of autumn to spring (e.g. *Poa annua*, *Stellaria media* agg., and *Capsella bursa-pastoris*), non-native species occupy this zone in summer (e.g. *Digitaria sanguinalis, Portulaca oleracea,* and *Eragrostis minor*). Most of these non-native species use C_4 or CAM photosynthetic pathways to utilize carbon dioxide. These pathways enable them to reach greater maximum quantum yield under higher temperatures and efficiently use water (Sage & Kubien 2003). Consequently, they have a competitive advantage under drier and warmer environmental conditions (i.e. above the pipeline) over species using C_3 photosynthetic pathway (Sage & Kubien 2003). However, Pearcy et al. (1981) suggested that not only high water use efficiency, but rather its interactions with rooting system and drought tolerance may advantage water-stressed C_4 species. Similar pattern switching from C_3 and C_4 vegetation between seasons was observed in the Great Plains of North America (Monson *et al.* 1983; Tieszen *et al.* 1997) and in the Sonoran desert (Mulroy & Rundel 1977) where dry and warm summer occurs (Barbour & Billings 1988). Thus, the temperature and moisture regimes on the studied pipeline can be tentatively analogized with those in the mentioned habitats.

Additionally, most of non-native species dominated above the pipeline in summer are archeophytes (*Digitaria sanguinalis, Setaria glauca, and Eragrostis minor*) and some are neophytes (*Porlutaca oleracea, Geranium pyrenaicum)* according to Catalogue of alien plants of the Czech Republic (Pyšek *et al.* 2012). Compared to above the pipeline, non-native species were much less represented outside the pipeline. It suggests that the zone above the pipeline represents a hot spot where non-native or even invasive species might grow and reproduce. Moreover, this hot spot is situated within a larger urban heat island which is known to harbour non-native species and to promote their invasions to other ecosystems (Oke 1982; Lonsdale 1999; Duguay *et al.* 2006; Pyšek *et al.* 2012).

Leaking heat from the pipeline significantly affects composition of vegetation, but also accelerates critical phenological events such as bud burst and flowering. Temperature is considered to be a key factor controlling important physiological processes, among others phenology (Saxe *et al.* 2001), and advance in phenological events is a well known response to increasing temperature (Arft *et al.* 1999; Natali *et al.* 2012). Thus, phenological shifts recently observed for various species are commonly attributed to temperature changes (Menzel *et al.* 2006; Khanduri *et al.* 2008). However, evaluation of these phenological shifts above the pipeline should be considered as preliminary, as not many species are shared between the pipeline and surrounding zones. Temperature gradient in close distance to the pipeline seems as more suitable place for such an observation. However, more detailed temperature data than currently available would be needed to reliably evaluate phenological changes associated to this zone.

Taking into account unusually high soil temperatures accompanied by rapid loss of soil moisture during the whole year, the zone above the pipeline can be considered as an extreme habitat for most of plant species native to the Czech Republic. Plants have to cope with desiccation in summer when vegetation cover on some plots decreases to 0%. In winter, snow layer rapidly thaws above the pipeline and aboveground plant tissues would have to cope with freezing air temperatures. Most of species commonly growing above the pipeline avoid these winter conditions through annual life cycle. Only a few annuals and biennials prosper on the pipeline zone in winter.

Numerous studies have been recently dedicated to experimental manipulations of climate in order to find out how are/will be different world ecosystems affected by climate change (Rustad *et al.* 2001; Wu *et al.* 2011; Michelsen *et al.* 2012). Environmental factors such as temperature (Harte & Shaw 1995), precipitation (Le Roux *et al.* 2005), atmospheric CO_2 (Williams *et al.* 2007) and nitrogen deposition (Dukes *et al.* 2005) have been manipulated either individually (Ziska 2003; Kudo *et al.* 2010) or combined (Zavaleta *et al.* 2003; Henry *et al.* 2005).

The impact of elevated temperature on vegetation structure and various plant traits has been widely investigated by field manipulations (Harte & Shaw 1995; Boeck *et al.* 2007; Yang *et al.* 2011; Natali *et al.* 2012; Buizer *et al.* 2012). Despite of number of experiments carried out, none of the experiments can be used as a straight-forward comparison to this study because of differences in heating method, intensity of heating, period of exposure and ecosystem studied.

Warming treatment in published studies was usually produced by one of four following heating facilities: overhead infrared heaters (Hovenden *et al.* 2008; Luo *et al.* 2010), open-top chambers (OTCs) (Jägerbrand *et al.* 2009; Sierra-Almeida & Cavieres 2010), night-time roofs (Sowerby *et al.* 2005; Sardans *et al.* 2008) or heating cables

(Akinola *et al.* 1998; Hartley *et al.* 2007). Overhead heaters are suspended 1-2.5 m above the soil surface and heat both air and soil (Harte *et al.* 2006). OTCs also manipulate air and soil temperatures (Dabros *et al.* 2010). In contrast to the previous devices, heating cables are placed at soil surface (Grime *et al.* 2008) or more frequently buried few centimetres underground (Hartley *et al.* 2007) and directly change soil temperature (Shen & Harte 2000). Other heating facilities were used scarcely (Beierkuhnlein *et al.* 2011). Manipulating temperature by heating cables is the most similar method to this study using pipeline-induced soil heating. Heating cables were used for the first time by Rykbost *et al.* (1975). Cables were buried 92 cm underground and increased the soil surface temperature by 3 °C. Purpose of the study was not to simulate climate change, but to validate feasibility of conveying waste water from power plants (25-40 °C) in pipelines under fields to increase crop yields.

The intensity of heating makes further comparisons almost impossible. While temperature near the soil surface was maintained in the majority of studies at about 1-3 °C above ambient temperature (Grime *et al.* 2000; Harte *et al.* 2006; Williams *et al.* 2007), the pipeline increases temperature at soil surface layer by more than 10 °C.

To distinguish heat-induced changes, the period of exposure of focal community to warming is crucial. In most of published studies, localities were heated for less than 10 years (Suttle *et al.* 2007; Peñuelas *et al.* 2007; Rollinson & Kaye 2012) and to my best knowledge, there are only two published studies with longer heat treatment (15 and 13 years; Harte *et al.* 2006; Grime *et al.* 2008). Moreover, warming treatment was applied in some cases only for a part of year (Grime *et al.* 2000; Dukes *et al.* 2005) or day (Sowerby *et al.* 2005; Sardans *et al.* 2008). Vegetation above the pipeline has been continuously heated since 1980s, which notably extends time used for heat treatment.

Warming experiments focused on urban lawn communities are very rare. Bijoor et *al.* (2008) examined impact of elevated temperature and fertilizer application on composition and nitrogen cycling of turfgrass (*Schedonorus phoenix*) lawn in Irvine, California. The lawn was heated about 4 °C by ceramic infrared heaters elevated 1 m above the soil surface and warming lasted for almost 1.5 years. Substantial increase in biomass of C_4 weed in heated plots was found, which strongly agrees with my observations of C_4 species abundance above the pipeline. Furthermore, C_4 weed rapidly increasing biomass was crabgrass (*Digitaria* sp.), which also dominated experimental plots above the pipeline during summer season. White *et al.* (2000) similarly showed that extreme heating event may favour C_4 grasses. In their study, three New Zealand perennial grasslands were treated by 8h-exposure to more than 50 °C by a special computer-regulated chamber. The exposure resulted in an increase of C_4 species abundance by 41 % in communities containing both C_3 and C_4 species. With absence of C_4 species, productivity of exposed C_3 community drastically decreased suggesting important role of C_4 species in maintaining stability of community under short-term extreme events. Removal of either C_3 or C_4 grass from the grassland ecosystem influenced many various attributes of that ecosystem (Wardle *et al.* 1999). Although other studies consistently proved promoting C_4 species under experimentally elevated temperatures (Wan 2005; Sherry *et al.* 2008), Sherry *et al.* (2008) pointed out that C4 species are not always facilitated by increased temperature and drought compare to C3 species (Williams *et al.* 2007).

4.2 Soil temperature and moisture

Great difference of soil temperature measured above and outside the pipeline suggests massive heat leakage from the pipeline. Not only soil temperature, but also soil moisture is strongly influenced by leaking heat. Therefore, it could be assumed that zone above the pipeline is permanently warmer and drier compared to surrounding. Nevertheless, winter temperatures were not always highest immediately above the pipeline (Fig. 14, 17). These particular temperature decreases might be caused by rapid thawing of snow layer in this zone followed by loss of its isolation properties. In addition, the pipeline is not deposited at all the sites in the same depth which may explain slightly lower temperatures measured at site 2. Detected steepest temperature gradient between the pipeline and most remote plots may be caused by differences in vegetation cover, soil properties or inaccurately delimited distance from the pipeline.

4.3 Germination of studied species

Overall proportion of germinated seeds in the laboratory and in the field was highest among all studied species in the case of *G. ciliata.* Most seeds (80-100%) germinated under temperature gradient at 22-28 °C, but similarly high proportion of seeds reached germination within preliminary germination trial at 21 °C. These results are in agreement with Jursík *et al.* (2003), who found out even wider range of optimum germination temperature (12-28 °C) with germination rate of 85%. Moreover, they demonstrated that germination significantly decreases at 30 °C, which is also supported by this study. On the other hand, germination rate of seeds of *G. ciliata* in a field experiment was much lower (30%). This can be attributed to strong dependence of germination of the species on soil temperature and moisture (Jursík *et al.* 2010), but other environmental factors such as light or disturbance can be also responsible for decreased germination in field experiments (Sweet 1986; Bernardová 2006). On the other hand, dormancy cannot be taken into account, as seeds of *G. ciliata* are primary dormant at most for 100 days after ripening (Jursík *et al.* 2003) and used seeds already overcome this period.

Compared to *G. ciliata*, *T. procumbens* is more inhibited by suboptimal temperatures and thus it optimally germinates only at temperatures ranging from 25-35 °C (Gyuimarães *et al.* 2000; Chauhan & Johnson 2008). Maximum proportion of its seeds germinated in my experiments at about 30 °C strongly supporting the previous finding but it should be mentioned that no higher temperature than 30 °C was possible to arrange in my experiment. While seeds exposed to temperature lower than 20 °C failed to germinate in this study, seeds in the study made by Gyuimarães *et al.* (2000) achieved germination rate of 5.4% at 20 °C and 1.3% at 15 °C. Such a low percentage of germination at lower temperatures may explain why no of 50 seeds subjected to 21 °C in preliminary trial germinated. Under field conditions, germination considerably declined with only 5% of sown seeds emerged. Dormancy of *T. procumbens* can be hypothesized as a reason of lower field emergence, but it is not known if seeds are dormant beyond its native range. In contrast, it is known that the species is highly sensitive to light and germinates predominantly from the surface layer (Chauhan & Johnson 2008).

S. inaequidens showed an ability to germinate in wide range of temperature (5-30 °C) with highest germination rate at about 18 °C. Wide range of temperatures suitable for germination of the species was already reported in several studies (Sans *et al.* 2004; López-García & Maillet 2005). Rapidity of germination observed by López-García & Maillet (2005) is also consistent with results of this study. Overall field germination rate was found to be about 50% lower than the germination rate observed in laboratory experiment. In contrast to the previous species, *S. inaequidens* is known to be dormant (Ernst 1998). According to López-García & Maillet (2005), the species can pose different levels of dormancy which prevent germination and emergence. Similar trend was observed for other weedy species (Bouwmeester & Karssen 1993).

Germination of *S. jacobaea* reflected similar wide range of temperature and similar optimal temperature as *S. inaequidens*. Recognized temperature range fully agree with results of Van der Meijden & Van der Waalskooi (1979) and optimal temperature was 3 °C lower in their study than detected here. Little bit different results with highest and fastest germination at 20/30 °C (day/night) were gained by Baker-Kratz & Maguire (1984). Cameron (1935) noticed rapid germination rate of the species at 15 °C. Although overall proportion of seeds germinated under temperature gradient was doubled compared to *S. inaequidens,* only 2% of sown seeds emerged in a field. It could be caused by a seed dormancy invoked by dessication of soil surface layer, where seeds occured (Sheldon 1974).

Although overall proportion of germinated seeds of *S. viscosus* was similar to *S. inaequidens*, it was achieved at much higher temperatures (27-30 °C). I assume that this result can be an artifact caused by delayed germination start of this species when compared to the rest of species. As germination velocity increased with increased temperature, only seeds subjected to higher temperature had enough time to germinate. Contrary to quite high overall germination rate under temperature gradient, almost no seeds germinated in a field experiment. Many irregular environmental factors such as temperature, light and moisture might have contributed to this decreased germination rate (Fenner & Thompson 2005). General absence of germination studies devoted to this species complicates to specify exact reason for the lack of germination and further data would be needed.

4.4 Comparison of germination and survival between zones

Among all studied species, only *T. procumbens* was found to germinate differently between zones. Successful germination together with an advance in phenology and longer survival of the species was significant above the pipeline. Since the length of the season is main factor limiting the number of produced seeds (Fenner & Thompson 2005), prolonged growing season of *T. procumbens* above the pipeline may be essential for establishment of the species. However, its establishment can be properly evaluated only after much longer time period (Turnbull *et al.* 2000; Clark *et al.* 2007).

No difference in germination of remaining species between zones might be related to the fact that they already occur in the Czech Republic (Pyšek *et al.* 2012). Therefore they are probably adapted to local climate and are able of establishment without any support (e.g. of elevated temperature). In contrast, *T. procumbens* is thermophilic species invading areas with tropical and subtropical climate, but recently not reported from Europe (DAISIE 2009).

5. Conclusions

A two-year investigation of vegetation above the pipeline proved that its composition is highly affected by heat leakage from the pipeline. Vegetation above the pipeline is characterized especially by annual, often non-native, species. Many of these species are C_4 grasses which deal with warm and dry conditions above the pipeline better than other species. Hypothesis considering zone above the pipeline as a heat island where non-native thermophilic, potentially invasive, plant species can recruit and establish was confirmed in a comparative study based on artificial sowing of closely-related native and non-native species.

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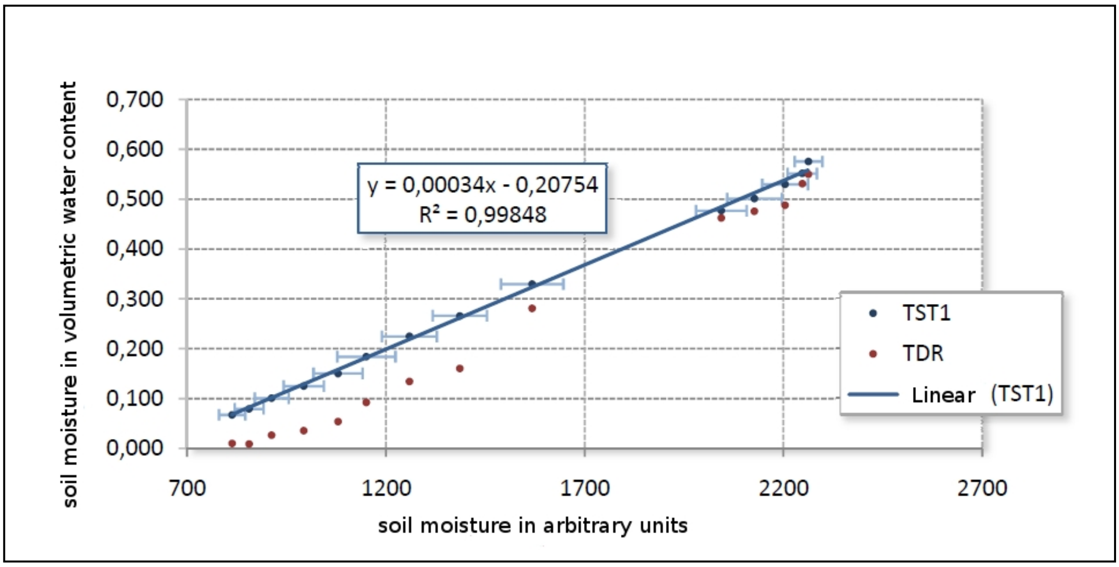
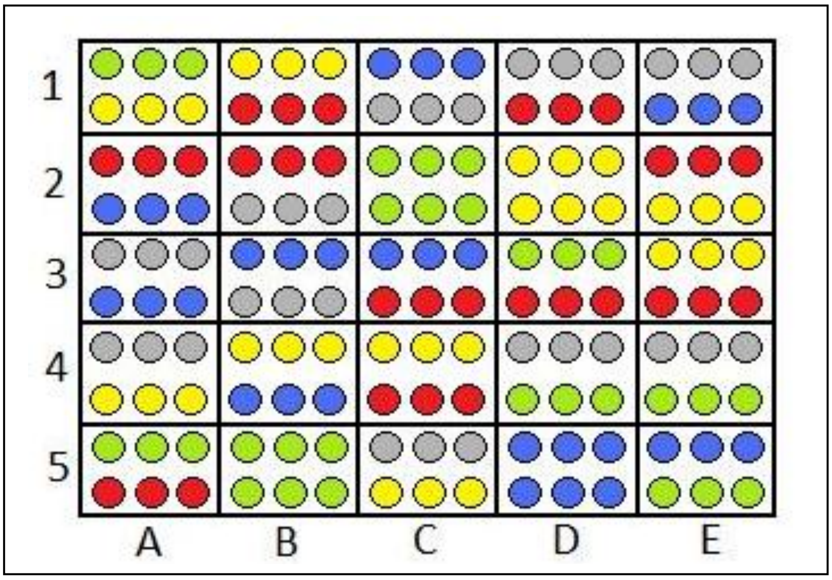
7. Supplementary Materials

Supplementary Material 1. Calibration curve used for converting values of soil moisture measured in arbitrary units (x axis) to the percentage of volumetric water content (m^3/m^3, y axis). TST1 correspond to used TMS dataloggers.



Supplementary Material 2. Random distribution of studied species in 25 six-well culture plates across the temperature gradient. Temperature decreases from A to E. Green color represents *S. inaequidens*, yellow *T. procumbens*, red *S. jacobaea*, blue *S. viscosus* and gray *G. ciliata*.



Supplementary Material 3. Species abbreviations used in the ordination diagrams.

AgroStol - Agrostis stolonifera, AjugRept - Ajuga reptans, AlopPrat - Alopecurus pratensis, AnemNemo - Anemone nemorosa, ArrhElat - Arrhenatherum elativ, BellPere - Bellis perennis, BromMoli - Bromus molis, CalaEpig – Calamagrostis epigejos, CapsBurs - Capsella bursa-pastoris, CeraGlom - Cerastium glomeratum, CeraHolo - Cerastium holosteoides, CirsArve - Cirsium arvense, DactGlom - Dactylis glomerata, DigiSang - Digitaria sanguinalis, EchinCrus - Echinochloa crus-galli, ElytRepe - Elytrigia repens, EragMino - Eragrostis minor, ErigAcri - Erigeron acris, ErodCicu - Erodium cicutarium, FestPrat - Festuca pratensis, FestRubr - Festuca rubra, GaliCili - Galinsoga ciliata, GaliAlbu - Galium album, GaliApar - Galium sarine, GeraPuss - Geranium pussilum, GeraPyre - Geranium pyrenaicum, GeumUrba - Geum urbanum, GlechHede - Glechoma hederacea, HypePerf - Hypericum perforatum, HypoRadi - Hypochaeris radista, ChenoAlbu - Chenopodium album, LactSerr - Lactuca serriola , LamiPurp - Lamium purpureum, LeonAutu - Leontodon autumnalis, LeonHisp - Leotodon hispidus, LoliPere - Lolium perene, MalvNegl - Malva neglecta, MatrDisc - Matricaria discoidea, MediLupu - Medicago lupulina, OxalCorn - Oxalis corniculata var. Repens, PersLapa - Persicaria lapathifolia, PhlePrat - Phleum pretense, PlanLanc - Plantago lanceolata, PlantMajo - Plantago major, PoaAnnua - Poa annua, PoaPrate - Poa pratensis, PoaTrivi - Poa trivialis, PolyAvic - Polygonum aviculare, PortOler - Portulaca oleracea, PoteRept - Potentilla reptans, PrunVulg - Prunella vulgaris, RanuRepe - Ranunculus repens, RobiPseu - Robinia pseudoacacia, RorrAust - Rorripa austriaca, RumeAcet - Rumex acetosa, RumeObtu - Rumex obtusifolius, SetaGlau - Setaria glauca, SisyOffi - Sisymbrium officinale, StelMedi - Stellaria media agg., Tara - Taraxacum sect. *Ruderalia, TrifPrat - Trifolium pretense, TrifRepe -Trifolium repens, TripInod - Tripleuspermum inodorum, UrtiDioi - Urtica dioica, VeroArve - Veronica arvensis, VeroHede - Veronica hederifolia* agg., *VeroChama - Veronica chamaedris, VeroOffic - Veronica officinalis, VeroPers - Veronica persica, ViciCracc - Vicia cracca*

Supplementary material 4. Photos.

Fig. 1. Study site 1 and 2.

Fig. 2. Study site 3.

Fig. 3. Temperature gradient table.

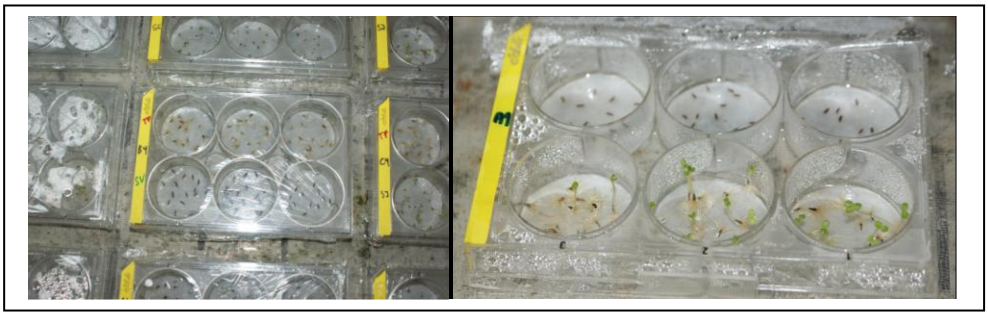


Fig. 4. Detail of six-well culture plates used for germination of studied species under the temperature gradient.

Fig. 5. Sowing plots above and outside the pipeline.

Fig. 6. Detail of sowing plots above the pipeline (upper) and outside the pipeline (bottom).

Fig. 7. Seedlings of studied species *(S. jacobaea, T. procumbens, G. ciliata, S. inaequidens)* in a field experiment.

Fig. 8. Flowering *G. ciliata*, *T. probumbens*, and *S. viscosus*.