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ORIGINAL PAPER

Searching for a universal indicator of plant stress: a three-year study of three woody species in three environmental gradients in boreal forests

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Abstract Stress in plants refers to adverse changes in their functioning. The occurrence and intensity of a stress can be assessed by alterations in plant traits, termed stress indicators. The ultimate goal of this study was to test whether six morpho-physiological plant traits, frequently used as stress indicators, respond consistently across species to various environmental stressors, with the aim of detecting universal stress indicators in forest tree species. We examined changes in vertical increment, leaf/needle size, shoot length, needle longevity, photosynthetic efficiency and fluctuating asymmetry in three common European tree species, mountain birch (*Betula pubescens* var. *pumila*), Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*) along three environmental gradients (elevation, pollution and seashore) from forests to stressful open environments. Data were collected in 2003, 2004 and 2005 from 297 trees growing naturally across 36 sites in north-western Russia. Fluctuating asymmetry was the only trait that did not vary among sites with differing levels of environmental stress. Leaf/needle size and shoot length occasionally changed along stress gradients, but the magnitude and direction of these changes differed by gradient type and species, resulting in no significant overall stress effect for either trait. In contrast, photosynthetic efficiency, vertical increment and needle longevity consistently decreased from low-stress to high-stress sites. The overall effect was significant for each of these three traits despite the magnitudes of these decreases differed depending on the gradient type and location, species, study year and individual tree. Replication at spatial, temporal and taxonomic levels ensured the robustness and reliability of our results that photosynthetic efficiency, vertical growth and needle longevity reliably captured a general stress syndrome and may serve as stress indicators in forest species.

Keywords Environmental stress gradients · Fluctuating asymmetry · Leaf size · Photosynthetic efficiency · Shoot length

Introduction

The term 'stress' is widely, albeit inconsistently, used to denote any deviations in an organism's functioning from its optimal state caused by the impacts of various stressors (Odum 1985). While the concept of stress is intuitively understood, plant stress is not clearly delineated (Bussotti and Pollastrini 2021) and its response-based definitions usually point to one or more stress indicators (Kranner et al. 2010). These indicators are crucial for assessing ecosystem health and the vulnerability of ecosystem components to climate change, land use, pollution and other environmental stressors (De Marco et al. 2022).

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Environmental gradients are widely utilized to identify plant traits potentially suitable as indicators of stress. Pollution, elevation and seashore gradients share common characteristics, as plant communities typically shift along these gradients in a consistent and predictable manner, from dense, tall forests in low-stress sites to sparse, stunted and low-stature trees in high-stress sites (Ruotsalainen et al. 2009). The morpho-physiological changes in plant traits along these gradients could be shaped by both common (e.g., increased wind speed) and specific stressors (e.g., UV-B in elevation gradients, heavy metals in pollution gradients, salt in seashore gradients), suggesting that some of the observed changes may be gradient-specific.

In plant ecology, the best-known concept of stress was introduced by Grime (1979), who characterised stress as a reduction in the rate of dry matter production caused by an external constraint. This rate is largely determined by the intensity of photosynthesis (Nowicka et al. 2018), which is significantly impaired by various environmental stressors (Lichtenthaler 1998; Brestic and Allakhverdiev 2022). Such impairment has made the maximum quantum yield of photosystem II (F_v/F_m) a widely used stress indicator (Daley 1995; Odasz-Albrigtsen et al. 2000). The trust in the suitability of chlorophyll fluorescence for plant stress assessment is so strong that devices for its measurement are sometimes called 'plant stress meters' (https://www.adc.co.uk/products/os30p-plant-stress-meter/). However, the published evidence on the impact of stressors on measures of photochemical efficiency is mixed, and interpretation remains complex (Bussotti and Pollastrini 2021). Several studies have reported a decline in chlorophyll fluorescence in plants growing in polluted areas (Odasz-Albrigtsen et al. 2000; Andreucci et al. 2006), at higher elevations (Miszalski et al. 2000) and near seashores (Naumann et al. 2008), while other studies did not detect decreases in F_v/F_m under the impacts of respective stressors (pollution: Kozlov et al. 2009; elevation: Bansal and Germino 2010; Reinhardt et al. 2011; salinity: Jimenez et al. 1997). Consequently, the question of whether a decrease in photosynthetic activity could be seen as a universal stress indicator remains unresolved.

Several morphological traits associated with plant growth and productivity, including (but not limited to) vertical and radial increments, shoot length, leaf size, needle longevity and crown defoliation, have been proposed as indicators for the non-destructive assessment of plant stress (Dässler 1976; Schubert 1985; Kullman 1996; Zarnoch et al. 2004; Kozlov et al. 2009). Tree growth is particularly widely used as an indicator of responses to various environmental stressors (Dobbertin 2005). It is typically assessed by repeatedly measuring tree diameter and/or height, which strongly correlate with wood volume and biomass (Bowman et al. 2013). Tree growth is significantly hampered with increases in pollution (Zvereva et al. 2010) and elevation (Kullman 1996), whereas no data are available from seashore gradients.

Changes in leaf/needle size and shoot length along environmental gradients are also variable. The meta-analysis of published data demonstrated a significant decrease in both traits with increasing industrial pollution (Zvereva et al. 2010), although several case studies failed to support this pattern (Kozlov et al. 2009). Leaf size showed no significant elevational variation (Midolo et al. 2019), whereas shoot length demonstrated variable elevational patterns, from decline (Gottardini et al. 2016; Wang et al. 2018) to the absence of changes (Cory et al. 2017) and even increase at higher elevations (Keleş 2020).

Needle longevity in conifers is considered as an important ecophysiological trait related to both carbon and nutrient balances (Aerts 1995). The effects of pollution on needle longevity have been known for decades (Treshow 1984), and a decrease in the number of needle age classes has often been suggested as one of the vitality indices for bioindication of pollution impact on forests (Dässler 1976; Schubert 1985). However, in environments with lower growing season temperatures (e.g., at higher latitudes and elevations), conifers tend to compensate for lower photosynthesis by higher longevity of needles (Ewers and Schmid 1981; Schoettle 1990). Effects of other stressors on needle longevity remain insufficiently known.

Among plant characteristics that are not functionally linked to productivity, fluctuating asymmetry (FA)—small, non-directional deviations from perfect symmetry in morphological traits (Zakharov 1990; Freeman et al. 1993)—is most widely used in environmental studies as an indicator of stress (Maldonado-López et al. 2019; Zakharov and Trofimov 2022), regardless of a steady accumulation of non-supportive evidence (Sandner and Matthies 2017; Gavrikov et al. 2023; Májeková et al. 2024). Variation in plant FA is well-studied along pollution gradients (Kozlov et al. 2009) but has been rarely explored along elevational gradients (Wilsey et al. 1998; Hagen et al. 2008) and seashore gradients (Cornelissen and Stiling 2011).

Despite the widespread use of the stress indicators listed above in forestry and environmental sciences, their responses to different stressors and across different tree species are quite variable. This variability may be at least partly explained by differences in research methodology in studies of plant stress that commonly address the responses of a single species to a single stressor (De Marco et al. 2022), raising uncertainty regarding whether the same indicator will work in other plant species or show a response to another stressor. This approach hinders the revelation of a universal indicator that would similarly respond to most, (if not all), of the stressful impacts in a majority of plant species. Comparing putative indicators, stressors and plant species within the same study, while ensuring the use of consistent techniques for data collection and processing, is crucial for identifying a universal indicator.

The ultimate goal of this study is to test whether six morpho-physiological plant traits frequently used as stress indicators respond consistently across species to various environmental stressors, with the aim of detecting universal stress indicators in forest trees. We compared changes in vertical increment, leaf size, shoot length, needle longevity, F_v/F_m and FA along three types of environmental gradients (elevation, pollution and seashore) in three forest-forming species widely distributed in Europe: mountain birch (*Betula pubescens* var. *pumila* (L.) Govaerts), Norway spruce (*Picea abies* (L.) Karst.) and Scots pine (*Pinus sylvestris* L.).

Materials and methods

Study region and species

This study was conducted on the Kola Peninsula in north-western Russia (Fig. 1). The region's climate is highly seasonal, with mean midwinter (January) temperatures ranging from –11 °C to –14 °C, midsummer (July) temperatures from + 11 °C to + 14 °C and annual precipitation between 450 and 560 mm. The summer is short, lasting for 2 to 3 months, with occasional snowfall even in midsummer, especially at high elevations.

The study encompasses three native species (mountain birch, Norway spruce and Scots pine), which together account for over 99% of the timber stock in the study region (faolex.fao.org/docs/pdf/rus155960(1).pdf). Mountain birch, a medium-sized deciduous species (Fig. 2c), is prevalent in the subarctic regions of Europe, particularly in Scandinavia and Iceland. It grows to 12–15 m tall with serrated leaves, 20–50 mm long, that turn yellow in autumn. This species is well adapted to harsh climates, demonstrating a high tolerance for cold temperatures and poor soils. Birch forests constitute 28% of the total forested area in the study region.

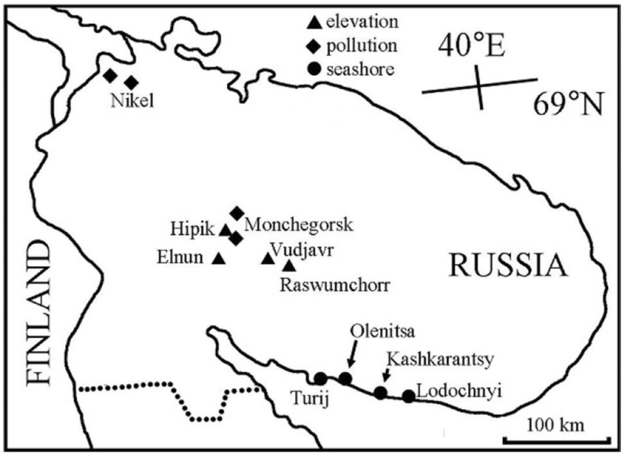

Fig. 1 Locations of environmental gradients on the Kola Peninsula, north-western Russia. For coordinates of study sites see Table S1

Norway spruce, a large evergreen conifer (Fig. 2a) native to Europe, grows in a range extending from northern Norway to the Carpathian Mountains. In the study region, it can grow up to 20 m in height with a conical crown, short, dark green needles and distinctive hanging cones. Spruce forests make up 29% of the total forested area in the study region.

Scots pine, an evergreen coniferous species (Fig. 2e), is native to Europe and Asia and is recognised by its tall, straight trunk and distinctive orange-red bark. It can reach heights of 25 m and has long blue-green needles. As a highly adaptable species, Scots pine grows in diverse habitats, ranging from coastal dunes to mountainous regions. It is a crucial timber species valued for its strong, durable wood, and pine forests account for 43% of the total forested area in the study region.

Environmental gradients

We investigated the environmental gradients imposed by: (1) differences in elevations in mountainous regions, (2) the effects of aerial pollution from copper-nickel smelters, and (3) proximity to the shores of the White Sea. Each environmental gradient was studied at four locations (Fig. 1) and included three sites representing high, moderate and low levels of environmental stress. For all gradients, the high-stress sites were located near the elevation, pollution and seashore tree lines, respectively, where sparse trees were spaced dozens of meters apart and exhibited stunted growth (Fig. 2b, d, f). The low-stress sites were chosen in continuous undisturbed forests closest to the high-stress sites (Fig. 2a, c, e), while the moderate-stress sites represented conditions intermediate between the high- and low-stress sites. Thus, plant community characteristics (independent of stressor type) rather than specific measures of individual stressors were used as diagnostic features of high-, moderate- and low-stress sites within each type of environmental gradient.

Altitudinal differences between high- and low-stress sites in our elevation gradients ranged from 115 to 325 m and horizontal distances between these sites ranged from 1 to 4 km Table S1). These gradients are primarily driven by the temperature of the growing season but also include increases in other stressors such as wind and UV-B (Paulsen and Körner 2014). The high-stress sites 455 to 615 m a. s. l. had sparser ground vegetation, with a higher proportion of lichens compared to low-stress sites at 140 to 355 m a. s. l. (Fig. 2a, b). One of the elevational gradients (Hipik) located close to Monchegorsk was moderately polluted, with concentrations of nickel (the main metal pollutant emitted

Fig. 2 Growth form of study trees on high-stress and low-stress sites: **a**, **b** Norway spruce; **c**, **d** mountain birch; **e**, **f** Scots pine; **a**, **c**, **e** low-stress sites; **b**, **d**, **f** high-stress sites. Locations: **a** Raswumchorr; **b** Hipik; **c**, **d** Monchegorsk; **e**, **f** Lodochnyi. For locations, see Fig. 1

by study smelters) in birch folage declining from 104 µg g^{-1} in low-elevational sites to 57 µg g^{-1} in high-elevation sites (Ruotsalainen et al. 2009). Excluding this moderately polluted gradient from the analyses did not alter the observed elevational patterns (data not shown).

Pollution gradients (Fig. 2c, d) extended from industrial barrens near the smelters in Monchegorsk and Nikel to nearly undisturbed forests 15 to 45 km away at similar elevations (Table S1). These gradients have evolved from pristine forests due to the toxic impacts of smelter emissions, mainly sulphur dioxide and trace elements. At high-stress barren sites, nickel concentrations in birch foliage ranged 103–172 µg g^{-1}, whereas at low-stress sites it reached 13–24 µg g^{-1} (Ruotsalainen et al. 2009). High-stress barren sites were characterised by bare ground, stunted and sparsely distributed trees, and occasional patches of dwarf shrubs, contrasting continuous dwarf shrub cover in low-stress forest sites (Kozlov et al. 2009).

Seashore gradients (Fig. 2e, f; Table S1) were much shorter than the elevation and pollution gradients. In two of the seashore gradients (Turij and Kashkarantsy), high-stress sites were 3–10 m wide sandy belts along the shoreline, and featured sparse and diverse vegetation, including dwarf shrubs and herbaceous plants. In the other two gradients (Olenitsa and Lodochnyi), the high-stress sites were 20–50 m wide and covered by tundra-like vegetation dominated by dwarf shrubs. The low-stress sites were located in mixed forests 150–300 m from the shoreline and exhibited much lower sodium concentration in birch foliage than high-stress sites (22–148 and 233–1066 µg g^{-1}, respectively: Ruotsalainen et al. 2009). The average elevational difference between high- and low-stress sites in seashore gradients was approximately 4 m (Table S1).

All three of the studied types of environmental gradients—two natural and one anthropogenic—demonstrated similar changes in tree growth form (Fig. 2) and were characterised by strong increases in wind velocity (Ruotsalainen et al. 2009). The anthropogenic pollution gradients differed from the other two natural gradients by substantial increases in deposition of sulphur dioxide and trace elements, whereas seashore gradients showed an eightfold increase in sodium (and possibly other elements found in seawater). No consistent within-gradient variation was found in soil pH, soil water potential, or soil nitrogen concentrations (Ruotsalainen et al. 2009).

Traits related to productivity

In early summer 2003, we selected three individuals of each tree species at each of the 36 sites. Spruce was absent from the Nikel pollution gradients, and pine from the Raswumchorr elevation gradient, and this affected the total number of trees (297). These differences, along with the death and cutting of a few trees by 2005, explain the variations in the degrees of freedom among our statistical models. We selected relatively young individuals (30 years-old for birch and spruce and 20 years-old for pine) with a median height of 115–165 cm. These trees were located 10–50 m apart and displayed growth forms and vigour typical of their respective sites.

Height, length of lateral shoots, size of leaves/needles, maximum needle age (only in spruce and pine), chlorophyll fluorescence (only in birch and pine), and FA (see below for details) were assessed in late summer (mid-August in the study area) from 2003 to 2005 (Data S1–S5). The vertical increment was calculated from heights measured in 2003 and 2005 with a ruler to the nearest 10 mm. The lengths of 10 haphazardly selected current-year shoots (long shoots in birch) located up to 2 m above the ground at different sides of the crown were measured with a ruler to the nearest 1 mm. Leaf length in birch and needle length in pine were measured from samples collected for FA measurements (see below). Ten spruce needles from the measured shoots (one needle per shoot) were dried at 105 °C for 24 h and weighed to the nearest 0.1 mg. Maximum needle age was assessed by counting the annual nodes that bore at least one live needle in two first-order branches (i.e., branches arising from the stem) located on opposite sides of the crown.

Chlorophyll fluorescence was determined in three birch leaves and in three groups of pine fascicles from each tree. For birch, the largest leaf from the chosen short shoot was consistently selected. In pine, 5–10 current-year needle fascicles from the middle of the terminal shoot on first-order branches were collected. A lightweight leaf cuvette that ensured dark adaptation was placed on the collected leaves/needles, and the cuvettes were stored in a plastic box to minimise desiccation. At 15–20 min after sampling, the ratio of variable to maximum chlorophyll fluorescence (maximum quantum yield of photosystem II, F_v/F_m) yielded under artificial light (200 μmol photons m^{-2} s^{-1}) was measured under field conditions using a plant stress meter (Biomonitor, S.C.I. AB, Umeå, Sweden).

Fluctuating asymmetry

For birch, the largest undamaged leaf from 10 short shoots was always sampled. Pine needle bundles (10 per tree) were collected from the middle of three to five current-year shoots of first-order branches. Leaves and needles were affixed to sturdy paper and press-dried similar to standard herbarium specimens (Kozlov et al. 2009; Kozlov and Zverev 2018). The FA of spruce shoots was assessed in situ to avoid cutting shoots and disturbing the growth pattern. For these measurements, 10 apical whorls located on first-order branches were selected, each consisting of two opposite second-order lateral shoots (Kozlov et al. 2009).

For birch leaves, the length of the lamina and the width of its left and right halves halfway between the leaf apex and the base of the petiole, were determined to the nearest 0.5 mm, ensuring that the ruler was perpendicular to the midrib (Data S1). In spruce whorls, the lengths of the first-order shoot and the left and right second-order shoots were measured to the nearest 1 mm from the point of departure from the axis to the base of the terminal bud (Data S2). In pine fascicles, the length of the longer of the two needles was measured to the nearest 1 mm, and the absolute difference in length between the two needles was measured to the nearest 0.025 mm with a stereomicroscope scale (Data S3). For more details on the measurement protocol, see Kozlov et al. (2009). Each measurement was performed twice by different persons. If the absolute difference between the first and second measurements exceeded the measurement accuracy by more than fivefold, a third measurement was carried out by a different person to correct for occasional errors.

Measurements of birch leaves and pine fascicles were blinded with respect to the sample origin. In contrast, measurements of spruce whorls in situ, as well as measurements of tree heights (Data S4) and shoot lengths (Data S5), were not blinded because the levels of environmental stress at the study sites were evident from the vegetation characteristics and tree vigour. In this case, blinding was achieved by not informing the measurers about the study hypothesis.

The FA values for birch and spruce were calculated using the formula:

$$\mathrm{FA} = 2 \times \mathrm{abs}\,(\mathrm{WL} - \mathrm{WR})/(\mathrm{WL} + \mathrm{WR}), \tag{1}$$

where WL and WR are the measurements of the left and right halves of the same leaf or the left and right shoots of the same whorl. For pine, the equation was modified slightly, so that:

$$\mathrm{FA} = \mathrm{dL}/\max\,(\mathrm{LL},\ \mathrm{LR}), \tag{2}$$

where dL is the absolute difference in length between the two needles, and max (LL, LR) the length of the longer needle in a fascicle. This size correction was used due to the weak but highly significant correlation between the absolute difference in left and right trait measurements and the average trait size (data not shown). The validation analysis of FA is described in Text S1.

Data analysis

Variation in plant traits was explored using mixed-model analysis (SAS GLIMMIX procedure, Type III sum of squares; SAS Institute 2009), first for each tree species separately and then for all tree species combined. In the first set of models, the type of gradient (elevation, pollution, seashore), stress level (high, moderate, low) and their interaction were treated as fixed effects, while 12 locations nested within gradient types, individual trees (108 birches, 90 spruces, and 99 pines), study years (2003, 2004, 2005) and the tree-by-year interaction were treated as random intercept effects. Models addressing variation in vertical increments included the same fixed effects, but the random effects were limited to locations nested within the gradient types. In the second set of models, which addressed species interactions with the types of environmental gradients, we employed the same fixed effects, while treating tree species, study sites, study years and their interactions as random intercept effects. For the analysis of vertical increments, the random effects were limited to tree species. In all models, we adjusted the standard errors and denominator degrees of freedom according to Kenward and Roger (2009).

The sources of variation in the magnitudes of observed effects were further explored by means of meta-analysis. For this purpose, Hedge's *d* measures of the effect sizes (ESs) were calculated for each of the 48 trait × tree species × gradient type combinations as the differences between the mean values of the parameter in high and low stress sites divided by the pooled standard deviation and weighted by sample size. An effect was considered statistically significant if the 95% confidence interval of the bootstrap-corrected mean ES did not include zero. Variation in ES among classes of categorical variables was explored by calculating the heterogeneity indices (Q_B) and testing these against the χ^2 distribution (Gurevitch and Hedges 2001). All analyses were performed using random effects models.

Results

Variation among plant traits

The species-specific analyses of six plant traits in different environmental gradients yielded diverse results, as significant variations among stress levels were observed only in 23 of 48 trait × tree species × gradient type combinations (Fig. 3). Some of these traits exhibited different, sometimes opposite, patterns in the same tree species within 3 types of gradients and 12 gradients. For example, pine in the seashore gradients attained its maximum annual growth on moderate-stress sites, whereas shoot length and needle length increased from low-stress to high-stress sites. In contrast, needle longevity and the F_v/F_m value for pine growing in seashore gradients decreased from low-stress to high-stress sites, whereas FA showed no variation among sites with different stress levels.

Overall, the six studied traits responded differently to stress ($Q_B = 14.49$, d.f. = 5, $P = 0.023$), and only three traits (vertical increment, F_v/F_m and maximum needle age) at high-stress sites were significantly (albeit to a different extent: $Q_B = 8.29$, d.f. = 2, $P = 0.026$) reduced relative to low-stress sites (Fig. 4a).

Variation among stress levels and gradient types

Only three of the 16 statistical models demonstrated significant and consistent variation in studied plant traits among stress levels across three types of environmental gradients (i.e., the interactions between the stress level and gradient type were not statistically significant): maximum needle age in spruce and pine and F_v/F_m in birch (Table 1, Fig. 3). Eight of the 16 models (Table 1) showed significant interactions between stress level and gradient type (i.e., changes in plant traits varied across gradients). For example, shoot length in pine did not change with elevation but decreased with pollution and increased with proximity to the seashore (Fig. 3).

A meta-analysis of all six plant traits showed significant variation in their changes along environmental gradients ($Q_B = 7.95$, d.f. = 2, $P = 0.033$). However, the traits that consistently decreased with increasing stress (vertical increment, F_v/F_m, and maximum needle age) were found to change in a similar way across the gradient types (Fig. 4b; $Q_B = 0.01$, d.f. = 2, $P = 0.99$).

Variation among tree species

Birch, pine and spruce demonstrated idiosyncratic responses in all studied traits to different levels of environmental stress, as indicated by significant stress level-by-species interactions (vertical increment: $\chi^2_1 = 10.4$, $P = 0.0013$; leaf/needle size: $\chi^2_1 = 8.62$, $P = 0.0033$; shoot length: $\chi^2_1 = 13.4$, $P = 0.0002$; needle longevity: $\chi^2_1 = 23.8$, $P < 0.0001$; F_v/F_m: $\chi^2_1 = 4.35$, $P = 0.0370$; FA: $\chi^2_1 = 5.02$, $P = 0.0251$). However, meta-analysis showed similar changes for all these indicators in all study species along environmental gradients ($Q_B = 1.21$, d.f. = 2, $P = 0.59$). Exclusion of traits showing no overall response to stress (i.e., leaf size, shoot length and FA) did not change this observation (Fig. 4b; $Q_B = 2.21$, d.f. = 2, $P = 0.35$).

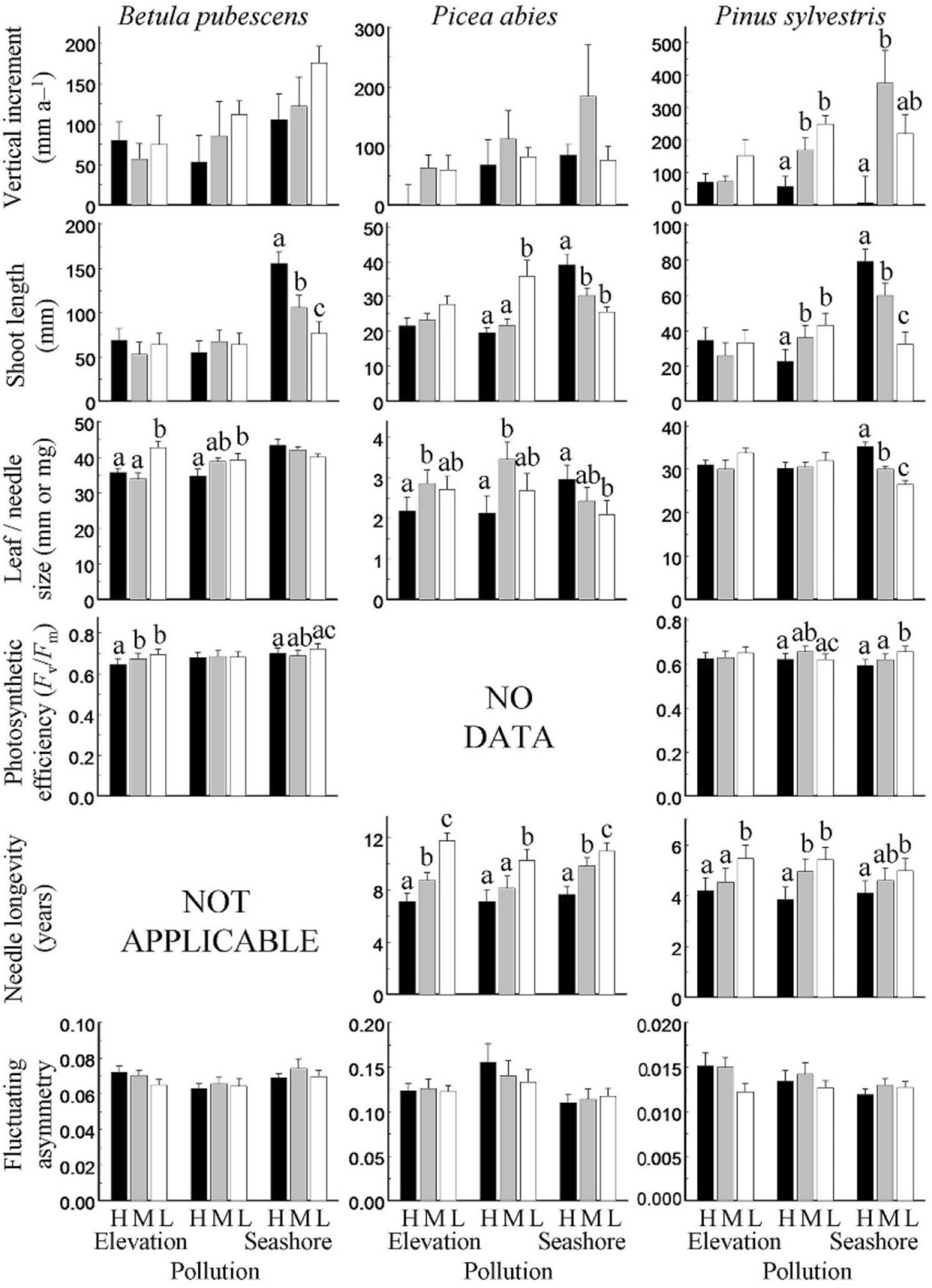

Fig. 3 Variation in plant traits among stress levels (H, high; M, moderate; L, low) within each type of environmental gradient. Values are means ± SE ($n = 12$ for each bar). Different letters indicate significant differences between stress levels (mixed model ANOVA, *t*-test); the absence of letters means no significant variation detected. 'No data' indicates that the trait was not measured due to technical limitations, whereas 'not applicable' refers to a trait that does not exist in the study species

Variation among locations, trees and years

Seven of the 16 statistical models revealed significant variation among locations (Table 1). Overall, 11 and 12 of 13 models confirmed significant variation among individual trees and study years, respectively. In seven models, the tree-by-year interaction was also significant. Intriguingly, significant variation was found among locations, trees and years for FA of all study species, in spite of the absence of significant variation among either stressors or stress levels.

Discussion

Universal indicatory traits

Only three of six commonly used morpho-physiological indicators of plant stress (photosynthetic efficiency, needle longevity and vertical increment) showed consistent differences between trees growing at the opposite ends of the three types of environmental gradients (elevation, pollution and seashore) in three common European forest trees (mountain

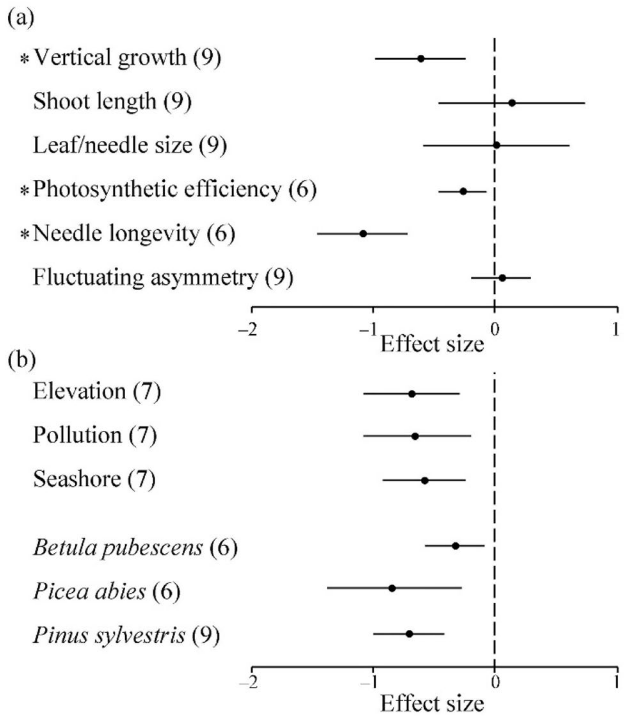

Fig. 4 Sources of variation in the magnitude of differences in putative stress indicators between high-stress and low-stress sites: **a** comparison of indicators; **b** comparisons of gradient types and tree species based on the three indicators that significantly responded to stress (marked by asterisks in panel **a**). Dots represent mean values; horizontal lines indicate 95% confidence intervals; sample sizes are shown in parentheses. A negative effect size indicates that the plant trait had lower values in high-stress sites compared to low-stress sites

birch, Norway spruce and Scots pine), and can therefore be tentatively regarded as universal indicators of stress. Consequently, comparison of indicatory properties was limited to these traits, addressing the criteria outlined by Dale and Beyeler (2001): ease of measurement, sensitivity, predictability, anticipatory, and low variability in response.

Photosynthesis, the process by which plants convert light energy into chemical energy, is particularly sensitive to environmental stress. Under stress, the photosynthetic apparatus becomes impaired due to oxidative damage, enzyme inhibition, and/or stomatal closure reducing CO_2 uptake (Chauhan et al. 2023). This leads to decreased electron transport and photochemical efficiency, observed as a reduction in F_v/F_m or CO_2 assimilation rate (Lichtenthaler 1998; Brestic and Allakhverdiev 2022).

Declines in F_v/F_m were consistently observed across our environmental gradients and tree species; however, mechanistic explanations of this consistency are lacking. An increase in wind velocity is the only abiotic stressor shared by study gradients (Ruotsalainen et al. 2009), but responses of chlorophyll fluorescence to wind varied from negative to positive across species (Clark et al. 2000). It is therefore suggested that molecular mechanisms leading to F_v/F_m decrease can be stressor-specific.

Furthermore, using this indicator in field conditions presents challenges. It requires costly equipment, and modifications are needed to adapt sensors for short needles of Norway spruce (a task which we were unable to perform with our instrument). Moreover, comparing locations accurately may necessitate repeated data collection over multiple growing seasons to account for tree-specific annual fluctuations in F_v/F_m (tree × year interaction in Table 1). Therefore, despite F_v/F_m is a potentially universal stress indicator, its practical use in field studies remains constrained by these logistical hurdles and variability of responses.

All woody plants periodically shed their leaves, with abscission regulated at the level of individual leaves. The timing of this process is governed by hormonal balance: ethylene promotes abscission, while auxin delays it (Roberts et al. 2002). An increase in ethylene production is a typical physiological response to abiotic stress and is commonly associated with the hardening or acclimation processes that plants employ to cope with such stress (Taylor and Whitelaw 2001; Chauhan et al. 2023).

The consistent decrease in needle longevity along all study gradients appeared somewhat surprising because, premature needle shedding was previously considered as a specific response to pollution (Kozlov et al. 2009) resulting from acceleration of the needle ageing processes (Wulff et al. 1996). However, our findings indicate that needle life span is affected by some stressor common for all our gradients. This may be wind velocity which was, on average, 3.5-fold greater in high-stress sites than in low-stress sites across all study gradients (Ruotsalainen et al. 2009). Strong winds accelerate needle surface wax erosion, especially in winter due to blowing snow, and this erosion likely plays a key role in limiting the elevational distribution of evergreen species (Hadley and Smith 1989). Measuring the maximum needle age in a tree with accessible branches takes only 1–2 min, making it a practical and integrative stress index for conifers, with an overall effect exceeding responses of other potential indicators, F_v/F_m and vertical increment, two- to five-fold (Fig. 4). However, data from pollution gradients in different climates suggest that the sensitivity of this indicator may decrease with rising mean annual temperatures (Kozlov et al. 2009), and broadleaved species have no equivalent index, limiting generalisations across species.

The typical trade-off between growth and stress resistance (Grime 1979) is attributed to limited resource availability. First, stress conditions reduce a plant's net carbon assimilation due to damage to the photosynthetic machinery (Chauhan et al. 2023). Second, stressed plants divert energy away from growth toward preventing or repairing stress-induced damage (Zhang et al. 2020). Consequently, stress-induced shifts in resource allocation—mediated by transcriptomic,

Table 1 Sources of variation in tree-specific morphological and physiological traits of three species (mountain birch, *Betula pubescens* var. *pumila*; Norway spruce; *Picea abies*; Scots pine, *Pinus sylvestris*) across three types of environmental gradients (elevation, pollution, seashore), each represented by four spatial replicates (locations nested within gradient types), with three levels of stress (high, moderate, low) during 2003–2005 (SAS GLIMMIX procedure, type III sum of squares)

Species	Effect type	Source of variation	Vertical increment		Shoot length		Leaf/needle size		Photosynthetic efficiency		Needle longevity		Fluctuating asymmetry	
			Statistics	*P*	Statistics	*P*	Statistics	*P*	Statistics	*P*	Statistics	*P*	Statistics	*P*
Birch	Fixed	Gradient type (GT)	$F_{2,9.2}=2.16$	0.17	$F_{2,9}=20.63$	0.0004	$F_{2,9}=4.24$	0.05	$F_{2,9}=1.21$	0.34	-	-	$F_{2,9}=2.79$	0.11
		Stress level (SL)	$F_{2,87.4}=1.63$	0.20	$F_{2,89.9}=10.96$	<.0001	$F_{2,90}=2.88$	0.06	$F_{2,89.8}=4.95$	0.0091	-	-	$F_{2,90}=0.82$	0.44
		GT×SL	$F_{2,87.4}=0.54$	0.71	$F_{4,89.9}=13.47$	<.0001	$F_{4,90}=5.31$	0.0007	$F_{4,89.8}=2.29$	0.0658	-	-	$F_{4,90}=0.52$	0.72
	Random	Location	$\chi^2_1=1.96$	0.16	$\chi^2_1=5.67$	0.0172	$\chi^2_1=2.35$	0.13	$\chi^2_1=27.09$	<.0001	-	-	$\chi^2_1=0.00$	0.96
		Tree	–	–	$\chi^2_1=2.32$	0.13	$\chi^2_1=114.6$	<.0001	$\chi^2_1=12.49$	0.0174	–	–	$\chi^2_1=5.66$	0.0174
		Year	–	–	$\chi^2_1=43.40$	<.0001	$\chi^2_1=81.1$	<.0001	$\chi^2_1=52.43$	<.0001	–	–	$\chi^2_1=6.59$	0.0103
		Tree × Year	–	–	$\chi^2_1=60.24$	<.0001	$\chi^2_1=5.26$	0.02	$\chi^2_1=279.48$	<.0001	–	–	$\chi^2_1=0.00$	0.96
Spruce	Fixed	Gradient type (GT)	$F_{2,6.1}=0.88$	0.46	$F_{2,7.0}=3.13$	0.11	$F_{2,7.1}=0.33$	0.73	–	–	$F_{2,7}=0.57$	0.59	$F_{2,6.8}=1.42$	0.91
		Stress level (SL)	$F_{2,63.3}=0.91$	0.41	$F_{2,72.9}=3.18$	0.0476	$F_{2,71.6}=2.89$	0.0622	–	–	$F_{2,74.1}=43.30$	<.0001	$F_{2,73.3}=0.09$	0.30
		GT×SL	$F_{4,62.9}=0.97$	0.43	$F_{4,73.1}=12.0$	<.0001	$F_{4,71.6}=3.78$	0.0076	–	–	$F_{4,73.7}=1.60$	0.18	$F_{4,73.6}=0.67$	0.62
	Random	Location	$\chi^2_1=7.76$	0.0053	$\chi^2_1=8.96$	0.0028	$\chi^2_1=1.73$	0.19	–	–	$\chi^2_1=11.23$	0.0008	$\chi^2_1=7.63$	0.0057
		Tree	–	–	$\chi^2_1=88.0$	<.0001	$\chi^2_1=175.79$	<.0001	–	–	$\chi^2_1=24.24$	<.0001	$\chi^2_1=0.77$	0.38
		Year	–	–	$\chi^2_1=60.8$	<.0001	$\chi^2_1=100.79$	<.0001	–	–	$\chi^2_1=0.03$	0.87	$\chi^2_1=9.45$	0.0021
		Tree × Year	–	–	$\chi^2_1=106.6$	<.0001	$\chi^2_1=0.00$	0.99	–	–	$\chi^2_1=0.00$	0.99	$\chi^2_1=0.00$	0.99
Pine	Fixed	Gradient type (GT)	$F_{2,8.3}=2.22$	0.17	$F_{2,8.1}=19.17$	0.0009	$F_{2,8.1}=0.58$	0.58	$F_{2,8.1}=0.51$	0.62	$F_{2,8}=0.20$	0.82	$F_{2,8.2}=1.26$	0.33
		Stress level (SL)	$F_{2,79.6}=7.42$	0.0011	$F_{2,81.2}=3.77$	0.0272	$F_{2,82.0}=1.45$	0.24	$F_{2,82.4}=3.80$	0.0264	$F_{2,80.5}=16.16$	<.0001	$F_{2,81.5}=1.77$	0.18
		GT×SL	$F_{4,79.6}=2.83$	0.0300	$F_{4,81.2}=19.77$	<.0001	$F_{4,82.0}=6.17$	0.0002	$F_{4,82.1}=3.72$	0.0079	$F_{4,80.4}=0.85$	0.50	$F_{4,81.4}=0.87$	0.49
	Random	Location	$\chi^2_1=0.00$	0.95	$\chi^2_1=1.92$	0.17	$\chi^2_1=0.67$	0.41	$\chi^2_1=13.79$	0.0002	$\chi^2_1=3.33$	0.0681	$\chi^2_1=0.83$	0.36
		Tree	–	–	$\chi^2_1=74.62$	<.0001	$\chi^2_1=36.4$	<.0001	$\chi^2_1=9.89$	0.0017	$\chi^2_1=14.76$	0.0001	$\chi^2_1=9.96$	0.0016
		Year	–	–	$\chi^2_1=94.17$	<.0001	$\chi^2_1=168.0$	<.0001	$\chi^2_1=34.06$	<.0001	$\chi^2_1=32.35$	<.0001	$\chi^2_1=19.7$	<.0001
		Tree × Year	–	–	$\chi^2_1=97.10$	<.0001	$\chi^2_1=41.1$	<.0001	$\chi^2_1=300.89$	<.0001	$\chi^2_1=0.00$	0.99	$\chi^2_1=0.00$	0.99

proteomic, and metabolomics changes in stressed plants (Shulaev et al. 2008; Cramer et al. 2011)—can alter crown architecture, particularly by hampering vertical growth of forest trees (Pearcy et al. 2005; Zverev et al. 2013).

Vertical tree increment showed an overall decrease with increasing stress (Fig. 3), despite extreme variability of this trait. This variability is likely attributable to the development of multiple leader shoots (Fig. 2b, d, f), which frequently replace each other due to the weakening of apical dominance in trees growing in high-stress habitats (Kullman 1996; Zverev et al. 2013). As a result, tree height can vary significantly between years, sometimes even showing negative vertical increments. Nevertheless, the consistent overall decrease in vertical growth with increasing stress levels across different gradient types suggests that this trait can be regarded as a universal indicator of stress conditions in trees.

Stressor-specific indicatory traits

In contrast to the traits discussed above, leaf size and shoot length exhibited stressor-specific changes: they decreased along pollution gradients, increased along seashore gradients, and remained unchanged along elevation gradients (Fig. 3), resulting in no overall effect across gradients (Fig. 4a). These findings corroborate the conclusion that changes in leaf size and shoot length in pollution gradients do not correlate with changes in chlorophyll fluorescence and needle longevity (Kozlov et al. 2009).

The decrease in leaf size and shoot length in our pollution gradients is likely driven by excess concentrations of nickel and copper in plants persisting in high-stress sites because adverse effects of these metalloids on these traits are well-known (Treshow 1984; Zvereva et al. 2010; Hassan et al. 2019). The absence of a significant overall effect of elevation on leaf size aligns with the results of a meta-analysis (Midolo et al. 2019), while the lack of elevational changes in shoot length falls within the range of variation reported in previous case studies (Gottardini et al. 2016; Cory et al. 2017; Wang et al. 2018; Keleş 2020). The increase in both leaf size and shoot length near the seashore is particularly surprising and counterintuitive, given that coastal areas are generally stressful for plants due to factors such as wind exposure, salt spray, soil salinity, dryness, high light intensity and nutrient deficiency (Hesp 1991). The decreases in vertical increment and chlorophyll fluorescence confirm that our plants experience stress near the seashore.

An increase in wind speed has been experimentally shown to reduce plant growth in general and leaf area in particular (Whitehead 1963). Consistently, the leaf size of the perennial herb *Farfugium japonicum* (L.) Kitam., naturally growing in coastal areas of Japan, decreased significantly with increased wind speed (Shiba et al. 2023). Moreover, the greatly elevated sodium concentration at high-stress seashore sites (Ruotsalainen et al. 2009) typically exerts adverse effects on plants (Na et al. 2015; Duan and Chang 2017). The adverse effects of the seashore environment on our study species are further shown by the two-fold greater mortality rate of mountain birch seedlings at the high-stress Olenitsa site relative to the low-stress Olenitsa site (Eränen and Kozlov 2009). We currently lack plausible mechanistic explanation for the observed increase in leaf and shoot size in our seashore gradients. This unexpected pattern challenges our understanding of plant responses to the multifaceted stressors associated with environmental gradients. We have concluded that the decrease in leaf and current-year shoot sizes is a specific plant response to stress caused by pollution, but these traits may not respond to other environmental stressors.

Non-indicatory trait

The developmental stability theory predicts that FA increases with both environmental and genomic stress (Møller and Swaddle 1997; Polak 2003). Over the past decade, attitudes of plant ecologists towards this putative stress indicator have shifted from uncritical acceptance to growing doubts, driven by the steady accumulation of inconclusive and negative results (Kozlov et al. 2009; Kozlov and Zverev 2018; Májeková et al. 2024). It is now evident that a significant proportion of previously published reports on a positive association between FA and stress, (or, more specifically, a measure of stressful impact), have resulted from research, confirmation and publication biases (Palmer 1999; Kozlov and Zvereva 2015; Kozlov 2017, 2024, 2025). In line with this conclusion, a rigorous analysis of our dataset, collected following best practices in FA studies (Graham 2021) and field ecology in general (Filazzola and Cahill 2021), clearly demonstrate that FA does not differ between stunted trees growing in high-stress open sites and their better-growing counterparts in low-stress continuous forest sites. The simultaneous exploration of several plant species across different types of environmental gradients, seen as a conceptual replication (as defined by Filazzola and Cahill 2021), gives particular weight to this discovery. Thus, our findings, in combination with other methodologically sound studies, fully support the conclusion that FA cannot be considered an indicator of plant stress (Kozlov 2017, 2025; Májeková et al. 2024).

However, FA is not merely statistical noise, as we revealed significant among-tree variation in birch and pine FA, whereas spruce FA varied among locations but not among trees. These findings suggest that FA level may result from a unique combination of environmental factors at each tree's location or from genetic influences that maintain consistent FA rankings in trees across different years. This consistency warrants further investigation, as it may facilitate the identification of FA drivers in plants. However, this work will only be effective if it is directed by a specific hypothesis, the development of which is currently hindered by an acute shortage of information on the eco-evolutionary significance of FA.

Conclusion

Our study is the first to compare changes in multiple traits of the same tree individuals across different environmental gradients and species, using uniformly collected multi-year data to ensure the robustness and reliability of our findings. The six morpho-physiological traits of study plants demonstrated variable changes within the explored environmental gradients. Among these, fluctuating asymmetry showed no variation among sites with differing levels of environmental impact across all gradients and species; thus, FA can not serve as a stress indicator. Leaf/needle size and shoot length occasionally changed along stress gradients, but the magnitude and even the direction of these changes varied by gradient type and species. Therefore, while these traits may indicate specific shifts in plant performance under certain conditions, they do not reliably capture a general stress syndrome defined as 'the symptoms of which are independent of the damaging agent' (Selye 1936). In contrast, the efficiency of photosynthesis, needle longevity and vertical tree growth consistently decreased from low-stress to high-stress sites across the three study species and across both natural (elevation, seashore) and anthropogenic (pollution) environmental gradients. Since these traits are functionally tied to dry matter production by the trees, their decreases align with Grime's definition of plant stress (Grime 1979). Therefore, we consider these traits to represent stress indicators in forest trees that can be used for early detection and monitoring of forest health across diverse environments and species, at least in the boreal biome.

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Data availability Data of this study are provided in the supplementary material.

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