Article

Variation in Leaf Size and Fluctuating Asymmetry of Mountain Birch (*Betula pubescens* var. *pumila*) in Space and Time: Implications for Global Change Research

Vitali Zverev and Mikhail V. Kozlov * [ID]

Department of Biology, University of Turku, 20014 Turku, Finland; vitzve@utu.fi
* Correspondence: mikoz@utu.fi

check for updates

Abstract: Experimental, latitudinal, and historical approaches have been used to explore and/or predict the effects of global change on biota, and each approach has its own advantages and disadvantages. The weaknesses of these individual approaches can, potentially, be avoided by applying them simultaneously, but this is rarely done in global change research. Here, we explored the temporal and spatial variations in the leaf size and fluctuating asymmetry (FA) of mountain birch (*Betula pubescens* var. *pumila*) in the Murmansk region of Russia, with the aim of verifying the predictions derived from the responses of these traits to experimental manipulations of abiotic drivers of global change. The examination of herbarium specimens revealed that leaf length increased during the 20th century, whereas the FA in the number of leaf teeth decreased, presumably reflecting an increase in the carbon and nitrogen availability to plants in that century. Along a northward latitudinal gradient, leaf length decreased whereas FA increased, presumably due to the poleward decreases in air temperature. The study site, collection year, and latitude explained a larger part of the leaf length variation in mountain birch relative to the variation in FA. Leaf length is likely a better indicator than FA in studies addressing global environmental change impacts on plant performance.

Keywords: *Betula pubescens* var. *pumila*; carbon dioxide; climate; environmental contamination; fluctuating asymmetry; herbarium specimens; Kola Peninsula; latitudinal gradient; leaf length; Murmansk region; nitrogen deposition

1. Introduction

Our environment is currently changing at an unprecedented rate. The increases in carbon dioxide (CO_2) concentration and ambient temperature, along with changes in precipitation, are among the most pressing environmental challenges facing the world today [1]. Consequently, much effort has been expended on characterizing the impacts of abiotic drivers of global change on biota. Hundreds of experiments, conducted in venues ranging from small-sized chambers to large free-air facilities, have explored the effects of CO_2, temperature, and precipitation on multiple organisms [2–5]. The main strength of this type of experimental approach is that it can often uncover cause-and-effect relationships between abiotic factors and biotic changes. However, global change affects more than just individual species because it reshuffles ecological communities, changes their composition, and alters species interactions. The consequences of these processes are difficult to explore experimentally, but their impacts on study organisms can be taken into account using data obtained from the natural ecosystems present in different climates [6] to verify the results of manipulative studies.

Future changes in biota can also be predicted from changes that have occurred in the past [7]. However, many abiotic factors that potentially affect biota, such as ambient temperature and CO_2

concentration, are strongly correlated at the time scale, thereby precluding the establishment of cause-and-effect relationships [8]. This correlation gives particular weight to data collected from regions that did not experience significant changes in climate for a certain period of time, despite continuous rises in CO_2 concentrations. One example of this type of region is the central part of the Murmansk area of Russia, where neither annual, seasonal, nor monthly mean temperatures showed any directional trend over the period from 1936 to 1998 [9]. Consequently, any changes in biota that occurred in this region between 1936 and 1998 should be attributable to factors other than temperature increases, particularly to the global elevation in CO_2 concentrations from 310 to 370 ppm (data from www.climate.gov).

We suggest that the weaknesses of the experimental, latitudinal, and historical approaches used to explore the effects of global change on biota can be avoided by applying these approaches simultaneously. Nevertheless, a combination of even two of these individual approaches is rarely used in global change research (but see [10,11]). In the present study, we explore naturally growing mountain birch (*Betula pubescens* var. *pumila* (L.) Govaerts) trees for both the temporal and spatial variation in two performance indices: leaf size and fluctuating asymmetry (FA hereafter). Our goal was to verify the predictions derived from experimental studies of their responses to CO_2 and temperature elevation with both historical and latitudinal data.

Leaf size is of a paramount importance for the carbon, water, and heat balance of a plant ([12] and references therein), and it frequently serves as a measure of plant vigor [13,14] or productivity [15]. Leaf FA, which is defined as small random deviations from perfect symmetry, was long considered a universal index of environmental and genomic stress [16–20], though the evidence is mixed on whether FA consistently and significantly increases in organisms facing unfavorable conditions during their development [21–25].

The experimental studies revealed increases in leaf size in *B. pubescens* [26,27] and in two other birch species [28,29] in response to elevated ambient temperature. The elevated levels of CO_2 increased the leaf size in *B. pubescens* [26], but did not induce changes in leaf size in two other birch species [30,31], although the leaf biomass in woody plants generally increased with CO_2 enrichment [2]. We are not aware of any experimental study that addressed the responses of leaf FA in *B. pubescens* to CO_2 and temperature elevation. However, warming decreased the leaf FA in *B. utilis* [28] and in *Vaccinium myrtillus* [32], whereas elevated CO_2 decreased the leaf FA of *Quercus geminate*, *Q. myrtifolia*, and *Myrtus communis* [33,34]. Based on the outcomes of these experiments, despite the absence of any temperature increases in our study region between 1935 and 1998 [9], we predict that we will find the increase in birch leaf size with the collection year during the 20th century, whereas the leaf FA will be larger in historical samples than in recent samples. At the spatial scale, we predict that the leaf size of mountain birch growing close to its northern distribution limit will decrease and the FA will increase from low to high latitudes due to the sharp decline in ambient temperature.

2. Materials and Methods

2.1. Study Object

Mountain birch is a tree-line variety of *B. pubescens* L., commonly known as downy birch. This deciduous, relatively small-leaved pioneer tree species is native and abundant throughout the boreal and temperate regions of Eurasia. In benign climates, it grows up to 25 m tall, but at the northern distribution limit, this species assumes a shrubby habit. The substantial ecological importance of mountain birch in subarctic forests has led to its intensive study in both pristine and disturbed environments [35–37].

2.2. Study Region

The study was conducted in the Murmansk region in northwestern Russia (Figure 1). The central and southern parts of this region are covered by Scots pine (*Pinus sylvestris* L.) and Norway spruce

(*Picea abies* (L.) Karst.) forests, whereas the northern part is dominated by dwarf shrub and lichen tundra. Both latitudinal (at about 69° N) and altitudinal (at about 350–450 m a.s.l.) tree lines are formed by sparse, low-stature woodlands of mountain birch. The field-layer vegetation in both forest and tundra sites consists primarily of crowberry (*Empetrum nigrum* ssp. *hermaphroditum* (Hagerup) Böcher), bilberry (*V. myrtillus* L.), and lingonberry (*V. vitis-idaea* L.).
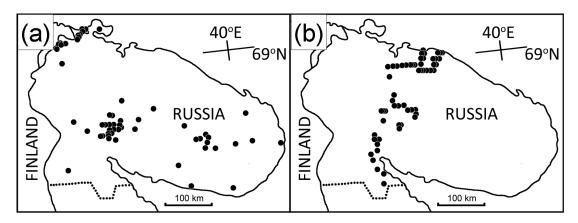

Figure 1. Sites of origin of mountain birch (*Betula pubescens* var. *pumila*) leaves in the Murmansk region: herbarium specimens collected in 1911-1965 (**a**) and trees sampled in 1996 (**b**).

The summer in our study region is cool and short, lasting from 2 to 3 months. The mean temperature in January in our sites ranges from −11 to −14 °C and in July from +11 to +14 °C, with an annual precipitation from 450 to 560 mm. The frost-free period varies from 50 to 100 days, but snow may occasionally occur even in midsummer.

2.3. Historical Samples

Natural history collections are a rich source of ecological and environmental information [38]. We searched for specimens of mountain birch collected before 1980 in the herbaria of the Komarov Botanical Institute (St. Petersburg, Russia) and the Avrorin Polar-Alpine Botanical Garden and Institute (Kirovsk, Russia). We discovered 76 specimens with fully grown leaves—i.e., those collected from July to September, but we disregarded two of them (collected in 1870 and 1977) as outliers on the time dimension. The exclusion of these outliers did not change the outcomes of the correlation analyses (data not shown). The remaining 74 specimens were collected from 1911 to 1965 across the entire Murmansk region, between the latitudes 66.2° and 69.7° N (Figure 1a). The year of data collection did not correlate with the latitude of the collection locality ($r = -0.05$, $n = 74$, $p = 0.66$).

2.4. Latitudinal Samples

The leaves of mountain birch were systematically collected in July 1996 along the roads connecting Murmansk and St. Petersburg, Revda, Teriberka, and Dalnie Zelentsy. The collection sites were selected in natural environments, at 20–50 km intervals in the forested part of the region and at 10–15 km intervals at the latitudinal tree line. The northernmost samples were collected from solitary birches persisting within the tundra. Despite the smaller longitudinal range relative to historical samples (Figure 1), the average locality coordinates were practically the same for latitudinal (68.2° N, 33.7° E) and historical (68.0° N, 33.8° E) samples. At each of the 94 sites, which were located between 66.5° and 69.2° N (Figure 1b), we haphazardly (on a "first found, first sampled" basis) selected five mature (aged over 20 years) individuals of mountain birch. From each selected birch, we collected five leaves, each from a different vegetative short shoot at a height of 0–2 m. We disregarded shoots with damaged leaves, and we always collected the largest leaf from the selected shoot. The leaves were mounted on strong paper using adhesive tape and then press-dried.

2.5. Measurements

In herbarium specimens, we measured the largest leaf from five vegetative short shoots haphazardly selected from different (whenever possible) parts of a specimen, disregarding shoots with leaves damaged by insects. Following the protocol described in our earlier study [37], we measured the length of each leaf lamina and the width of the left and right sides from the midrib to the leaf margins (at the midpoint between the base and the tip) perpendicular to the midrib. The measurements were conducted with a ruler to the nearest 0.5 mm; the perpendicularity of the measurement line to the midrib was controlled visually. Additionally, while keeping in mind the relatively low reproducibility of these measurements in birch [39], we counted the numbers of marginal teeth on the left and right sides of each leaf (Data S1 in the Supplementary Material). We repeated these measurements in a sub-sample of 100 randomly selected leaves to quantify the reproducibility of the results (Data S2 in the Supplementary Material).

2.6. Calculation of FA and Testing for Reproducibility

We calculated the FA values as follows: FA = $2 \times$ abs(L $-$ R)/(L $+$ R), where L and R are the values of the assessed character (half-width in FA_W and teeth number in FA_N) in the left and right leaf halves. This index, labelled FA2 by Palmer and Strobeck [40], was applied in many earlier studies of plant leaf FA [28,37,41]. The use of this relative index is justified by the significant positive correlation between the absolute difference in the assessed characters between the left and right leaf halves and their average values (FA_N: $r = 0.09$, $n = 2901$ leaves, $p < 0.0001$; FA_W: $r = 0.14$, $n = 2908$ leaves, $p < 0.0001$). The leaf length and two FA measures were only weakly, although significantly, correlated to each other ($R^2 = 0.0013$–0.0066, $p \leq 0.05$), indicating that the simultaneous analysis of these three performance indices is not redundant.

We tested for the presence of directional asymmetry and FA in a random subsample of 100 leaves by mixed-model ANOVA. In this analysis, we considered the leaf side a fixed factor and the individual leaf a random factor (procedure MIXED [42]). We evaluated the reproducibility of FA_N and FA_W by calculating the index ME5 = (MSi $-$ MSm)/(MSi $+$ (n $-$ 1) $\times$ MSm), where MSi and MSm are the interaction and error mean squares from a sides $\times$ individuals ANOVA, and n is the number of measurements [40]. This index expresses FA variation as a proportion of the total variation between the leaf sides, which includes variation due to both FA and measurement error.

2.7. Data Analysis

FA_W and FA_N were square-root transformed to meet a normality assumption, averaged for tree-specific values, and then analyzed with a linear mixed model (the GLIMMIX procedure [42]). Several samples, both historical and recent, originated from the impact zones of smelters located in Monchegorsk, Nikel, Zapolyarnyy, and Kandalaksha. Therefore, we first contrasted the FA values between the polluted and unpolluted localities. The effect of pollution on FA, in line with an earlier study [37], was far from being significant ($p > 0.30$); therefore, we excluded the pollution level from the further analyses. We considered the period of birch collection (historical vs. recent) as a fixed effect and the latitude of the collection site as a covariate, whereas the site of leaf origin was treated as a random intercept effect. We facilitated accurate *F* tests of the fixed effects by adjusting the standard errors and denominator degrees of freedom by the latest version of the method described by Kenward and Roger [43]. The significance of a random factor was evaluated by calculating the likelihood ratio and testing it against the chi-squared distribution [44]. The relationships of the birch performance indices with the latitude and collection year were quantified by calculating the Pearson product-moment correlation coefficients. The relative changes in the performance indices in space and time were estimated from linear regression models.

3. Results

The highly significant side × leaf interactions in the subsample of 100 leaves confirmed the existence of FA in both measured traits and verified our ability to identify this FA using measurements of the given accuracy (Table 1). The reproducibility of FA_N (ME5 = 0.59) was greater than that of FA_W (ME5 = 0.41).

Table 1. Basic statistics for the measurements of differences in leaf width and teeth number between the left and right sides in a random sample of 100 leaves of mountain birch (*Betula pubescens* var. *pumila*) (SAS GLM procedure, type 3 tests).

Source of Variation	Leaf Width		Teeth Number	
	Test Statistics	*p*	Test Statistics	*p*
Side	$F_{1,200} = 0.42$	0.52	$F_{1,200} = 25.5$	<0.0001
Individual	$F_{99,200} = 189.4$	<0.0001	$F_{99,200} = 238.0$	<0.0001
Side × Individual	$F_{99,200} = 4.85$	<0.0001	$F_{99,200} = 7.93$	<0.0001

The length and FA_N of the mountain birch leaves differed between the collection periods and showed different changes with latitude in the historical versus recent samples (Table 2). The leaves from the historical samples, on average, were slightly larger and had a higher FA_N compared with the leaves collected in 1996. By contrast, the FA_W did not differ between the historical and recent samples (Figure 2).

Table 2. Sources of variation in length and fluctuating asymmetry (FA) of mountain birch (*Betula pubescens* var. *pumila*) leaves (SAS GLIMMIX procedure, type 3 tests).

Source of Variation	Leaf Length		FA_N [†]		FA_W [‡]	
	Test Statistics	*p*	Test Statistics	*p*	Test Statistics	*p*
Date *	$F_{1,167.6} = 23.6$	<0.0001	$F_{1,402.2} = 4.73$	0.0301	$F_{1,361.6} = 0.58$	0.45
Latitude	$F_{1,167.8} = 62.3$	<0.0001	$F_{1,403.1} = 5.05$	0.0251	$F_{1,362.5} = 1.80$	0.18
Date × Latitude	$F_{1,167.8} = 23.7$	<0.0001	$F_{1,403.1} = 4.45$	0.0355	$F_{1,362.5} = 0.59$	0.44
Site	$\chi^2_1 = 159.9$	<0.0001	$\chi^2_1 = 2.53$	0.0560	$\chi^2_1 = 5.37$	0.0103

[†] FA_N: fluctuating asymmetry in the number of leaf teeth. [‡] FA_W: fluctuating asymmetry in leaf width. * Date: 1911–1965 vs. 1996.
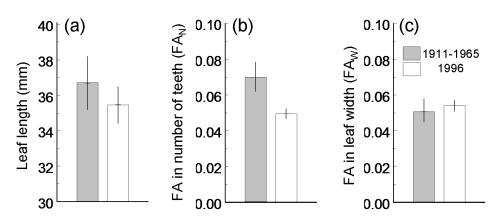

Figure 2. Estimated marginal means (±S.E.) of leaf length (**a**), fluctuating asymmetry in the number of leaf teeth (**b**), and fluctuating asymmetry in leaf width (**c**) of mountain birch (*Betula pubescens* var. *pumila*) during the two collection periods.

Within the historical samples, the leaf length increased by 25% between 1911 and 1965 (Figure 3a) and tended to decrease in a northward direction (Figure 4a), whereas none of the FA measures correlated with either collection year (Figure 3b,c) or latitude (Figure 4c,e). In 1996, the leaf length

decreased by 40% between 66.5 and 69.2° N (Figure 4b), whereas both FA$_N$ and FA$_W$ increased towards the north by 69% and 40%, respectively (Figure 4d,f).
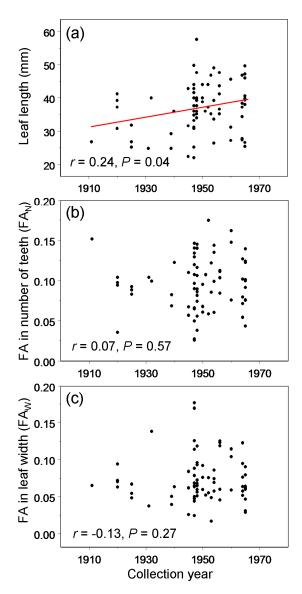

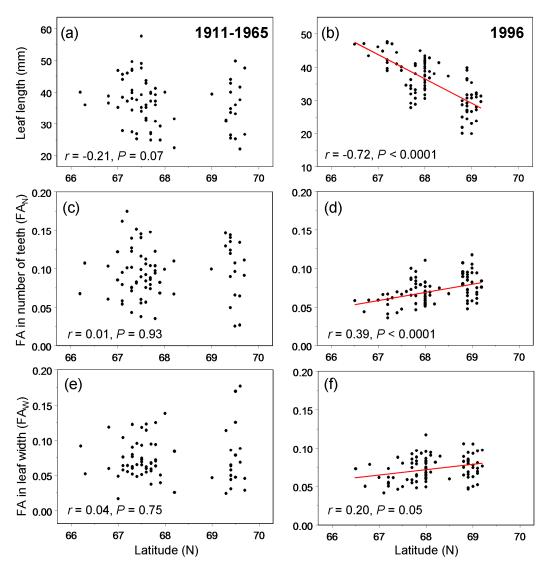**Figure 3.** Changes in leaf length (**a**), fluctuating asymmetry in the number of leaf teeth (**b**), and fluctuating asymmetry in the leaf width (**c**) of mountain birch (*Betula pubescens* var. *pumila*) between 1911 and 1965. Correlations are based on non-transformed values of leaf length and on square-root transformed values of FA$_N$ and FA$_W$.

Figure 4. Latitudinal changes in leaf length (**a**,**b**), fluctuating asymmetry in the number of leaf teeth (**c**,**d**), and fluctuating asymmetry in the leaf width (**e**,**f**) of mountain birch (*Betula pubescens* var. *pumila*) during the two collection periods, 1911–1965 (**a**,**c**,**e**) and 1996 (**b**,**d**,**f**). Correlations are based on non-transformed values of leaf length and on the square-root transformed values of FA_N and FA_W.

4. Discussion

4.1. Temporal Variation

In agreement with our prediction, the length of the mountain birch leaves increased between 1911 and 1965, presumably due to an increase in the ambient CO_2 concentrations during the 20th century. However, the plant growth in boreal forests is commonly constrained by a low supply of nitrogen [45], and the experimental addition of nitrogen increased the leaf size of both *B. pubescens* [28] and *B. pendula* [46]. Therefore, we cannot exclude the possibility that the fivefold increase in soluble nitrogen deposition that occurred in Europe between 1905 and 1995 [47] may have contributed to the increase in leaf length detected in herbarium specimens.

The leaf length of mountain birch in 1996 was 3.5% smaller than in 1911–1965. This result seemingly contradicts our finding of a positive correlation between leaf size and collection year. However, repeated observations of mountain birch growing in the central part of the Murmansk region demonstrated that the leaf size varied greatly among years and that the average leaf length from 1993–2010 was 42.2 mm [48]—i.e., much larger than the 36.7 mm determined for historical samples

(Figure 2a). Most likely, the exceptionally small size of birch leaves in 1996 was related to extremely low spring temperatures, which, in this year, attained the lowest value between 1991 and 2014 [49].

Only the contrast between FA_N in historical and recent samples confirmed the predicted decrease in FA during the 20th century due to an increase in the ambient CO_2 concentration. The FA_W did not differ between the historical and recent samples, and neither FA measurement showed a correlation with the collection year within the historical samples. This result suggests either that FA_N and FA_W show a different sensitivity to CO_2 elevation or that FA_N is better suited than FA_W for the analysis of relatively small samples due to its lower measurement error. Nonetheless, the data on leaf length and FA_N yielded consistent conclusions regarding the increase in mountain birch performance during the 20th century.

4.2. Spatial Variation

In line with our prediction, the length of mountain birch leaves collected in 1996 decreased and the FA increased towards the north. The poleward decline in ambient temperature is the likely driver of these changes, because FA was earlier found to decrease with temperature elevation both in experimental [28] and observational [37] studies of birches. This pattern may also result from the latitudinal increase in the seasonal temperature variation [50], but observational data do not allow us to uncover the cause-and-effect relationships behind the observed pattern. The absence of similar trends in historical samples most likely results from the high among-year variation in both the leaf size and FA [37,48], combined with the less accurate values of FA due to the lower sizes of historical samples relative to recent samples.

The increase in FA near the latitudinal distribution limit of mountain birch in northwestern Russia is consistent with the FA increase observed in this species at the altitudinal tree line in Norway [51] and in marginal populations of oaks in Florida [41]. However, this pattern cannot be seen as the general regularity, because the FA in the honey locust, *Gleditsia triacanthos* L., across the USA was neither increased in marginal populations nor correlated with latitude or climate [52].

The poleward decrease in the leaf size of mountain birch is consistent with earlier observations conducted on downy birch in Asia [15]. In several tree species, leaf size was positively correlated with the mean annual temperature and/or precipitation [11,53], although the absence of correlation with either latitude or climate had also been reported [54]. The likely reason for the poleward decline in the leaf size of mountain birch is the decrease in ambient temperature, because an experimental temperature elevation increased the leaf size in several birch species [26–29]. However, the weather conditions did not explain the observed among-year variation in leaf size of mountain birch growing in the central part of the Murmansk region [48], suggesting that other factors than change in temperature [6] may have contributed to the observed pattern.

4.3. Importance of FA Measurements in Plants

The underlying assumption of FA analysis is that the development of the two sides of a bilaterally symmetrical leaf is controlled by identical genes and that, as a consequence, non-directional differences between the sides result from stressful environmental impacts that have occurred during leaf growth [55]. This is a nice hypothesis—when it works. However, the evidence accumulated to date [22–25,56] suggests that support for this hypothesis is found in no more than half of the published studies. The actual proportion of opposing results may be even higher because of a preferential publication of confirmatory results [57,58].

Despite a huge amount of published information, it is still impossible to predict whether the FA in a particular trait of a particular species will change in response to a particular environmental factor. This problem cannot be resolved with observational data, which still dominate this research field. We therefore suggest that future studies of FA give more weight to the experimental approach. An exploration of the biological importance of leaf FA for plants [59] and for herbivores [60,61] may also shed light on the factors controlling FA at both the ecological and evolutionary time scales. In this

way, we can improve our understanding of the functional links between environmental stress and FA, if they do exist.

Coming back to our results, we ask the question: did FA analysis reveal any regularity that was not discovered from leaf length measurements? The answer is yes, but only in one particular case: The contrast between the historical and recent samples revealed the predicted pattern in FA_N but not in leaf length (Figure 2a,b). In all other analyses, FA either yielded the same conclusions as leaf length (e.g., in the latitudinal gradient; Figure 4) or showed no pattern when the changes in leaf length were significant (e.g., between 1911 and 1965; Figure 3). Overall, the leaf length demonstrated a stronger association with the explanatory variables and a larger variation among the study sites than was observed for either of the FA measures (Table 2; Figures 3 and 4). This finding is in line with the definition of stress as the reduction in plant growth under adverse conditions [62]. Based on this definition, leaf size is a direct measure of stress in plants, whereas FA is an indirect measure. Importantly, the measurements of plant growth are usually less laborious and more accurate than the measurements of FA, which raises further questions regarding the use of FA as a stress index at the level of plant individuals and populations.

5. Conclusions

The variation in the explored performance indices of mountain birch over space and time generally confirmed the predictions based on experimental studies. The performance of mountain birch growing close to the northern tree limit, as invoked from measurements of leaf length and FA_N, increased during the 20th century, presumably due to an increase in carbon and nitrogen availability to the plants, and it decreased with increases in latitude, presumably due to decreases in the ambient temperature. The consistency in the association of the leaf FA with the ambient temperature over years [37] and across latitudes (this study) demonstrated that the responses of FA to temperature elevation determined experimentally [28] provide an adequate prediction of the patterns occurring in natural ecosystems. The asymmetry in the number of leaf teeth (i.e., FA_N) showed a higher reproducibility and was better associated with the explanatory variables than the asymmetry in leaf width (i.e., FA_W) was. The study site, collection year, and latitude explained a larger part of the variation in the leaf length of mountain birch relative to the variation in FA. Therefore, leaf length is likely a better indicator than leaf FA in studies addressing the impacts of global environmental change on plant performance.

References

1. Intergovernmental Panel on Climate Change (IPCC). *Climate Change 2014: Impacts, Adaptation, and Vulnerability*; Cambridge University Press: Cambridge, UK; New York, NY, USA, 2014.
2. Curtis, P.S.; Wang, X.Z. A meta-analysis of elevated CO_2 effects on woody plant mass, form, and physiology. *Oecologia* **1998**, *113*, 299–313. [CrossRef] [PubMed]

3. Ainsworth, E.A.; Long, S.P. What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. *New Phytol.* **2005**, *165*, 351–372. [CrossRef] [PubMed]

4. Zvereva, E.L.; Kozlov, M.V. Consequences of simultaneous elevation of carbon dioxide and temperature for plant-herbivore interactions: A meta-analysis. *Glob. Chang. Biol.* **2006**, *12*, 27–41. [CrossRef]

5. Tylianakis, J.M.; Didham, R.K.; Bascompte, J.; Wardle, D.A. Global change and species interactions in terrestrial ecosystems. *Ecol. Lett.* **2008**, *11*, 1351–1363. [CrossRef]

6. De Frenne, P.; Graae, B.J.; Rodríguez-Sánchez, F.; Kolb, A.; Chabrerie, O.; Decocq, G.; De Kort, H.; De Schrijver, A.; Diekmann, M.; Eriksson, O.; et al. Latitudinal gradients as natural laboratories to infer species' responses to temperature. *J. Ecol.* **2013**, *101*, 784–795. [CrossRef]

7. Wolkovich, E.M.; Cook, B.I.; McLauchlan, K.K.; Davies, T.J. Temporal ecology in the Anthropocene. *Ecol. Lett.* **2014**, *17*, 1365–1379. [CrossRef]

8. Stips, A.; Macias, D.; Coughlan, C.; Garcia-Gorriz, E.; San Liang, X. On the causal structure between CO_2 and global temperature. *Sci. Rep.* **2016**, *6*, 21691. [CrossRef]

9. Kozlov, M.V.; Berlina, N.G. Decline in length of the summer season on the Kola Peninsula, Russia. *Clim. Chang.* **2002**, *54*, 387–398. [CrossRef]

10. Guerin, G.R.; Wen, H.; Lowe, A.J. Leaf morphology shift linked to climate change. *Biol. Lett.* **2012**, *8*, 882–886. [CrossRef]

11. Li, Y.; Zou, D.; Shrestha, N.; Xu, X.; Wang, Q.; Jia, W.; Wang, Z. Spatiotemporal variation in leaf size and shape in response to climate. *J. Plant Ecol.* **2020**, *13*, 87–96. [CrossRef]

12. Poorter, L.; Rozendaal, D.M.A. Leaf size and leaf display of thirty-eight tropical tree species. *Oecologia* **2008**, *158*, 35–46. [CrossRef] [PubMed]

13. Price, P.W. The plant vigour hypothesis and herbivore attack. *Oikos* **1991**, *62*, 244–251. [CrossRef]

14. De Bruyn, L.; Scheirs, J.; Verhagen, R. Nutrient stress, host plant quality and herbivore performance of a leaf-mining fly on grass. *Oecologia* **2002**, *130*, 594–599. [CrossRef] [PubMed]

15. Migalina, S.V.; Ivanova, L.A.; Makhnev, A.K. Size of the leaf as a marker of birch productivity at a distance from the climatic optimum. *Russ. J. Plant Physiol.* **2009**, *56*, 858–862. [CrossRef]

16. Zakharov, V.M. Analysis of fluctuating asymmetry as a method of biomonitoring at the population level. In *Bioindications of Chemical and Radioactive Pollution*; Krivolutsky, D., Ed.; Mir Publishers: Moscow, Russia, 1990; pp. 187–198.

17. Clarke, G.M. Fluctuating asymmetry: A technique for measuring developmental stress of genetic and environmental origin. *Acta Zool. Fenn.* **1992**, *191*, 31–35.

18. Parsons, P.A. Fluctuating asymmetry—a biological monitor of environmental and genomic stress. *Heredity* **1992**, *68*, 361–364. [CrossRef]

19. Graham, J.H.; Freeman, D.C.; Emlen, J.M. Antisymmetry, directional asymmetry, and dynamic morphogenesis. *Genetica* **1993**, *89*, 121–137. [CrossRef]

20. Freeman, D.C.; Graham, J.H.; Emlen, J.M. Developmental stability in plants—symmetries, stress and epigenesis. *Genetica* **1993**, *89*, 97–119. [CrossRef]

21. Palmer, A.R. Waltzing with asymmetry. *Bioscience* **1996**, *46*, 518–532. [CrossRef]

22. Anne, P.; Mawri, F.; Gladstone, S.; Freeman, D.C. Is fluctuating asymmetry a reliable biomonitor of stress? A test using life history parameters in soybean. *Intern. J. Plant Sci.* **1998**, *159*, 559–565. [CrossRef]

23. Graham, J.H.; Raz, S.; Hel-Or, H.; Nevo, E. Fluctuating asymmetry: Methods, theory, and applications. *Symmetry* **2010**, *2*, 466–540. [CrossRef]

24. Kozlov, M.V. Plant studies on fluctuating asymmetry in Russia: Mythology and methodology. *Russ. J. Ecol.* **2017**, *48*, 1–9. [CrossRef]

25. Sandner, T.M.; Matthies, D. Fluctuating asymmetry of leaves is a poor indicator of environmental stress and genetic stress by inbreeding in *Silene vulgaris*. *Ecol. Indic.* **2017**, *79*, 247–253. [CrossRef]

26. Mortensen, L.M. Effects of elevated CO_2 concentration on growth of *Betula pubescens* Ehrh. in different climatic conditions. *Scand. J. For. Res.* **1998**, *13*, 197–203. [CrossRef]

27. Grau, O.; Ninot, J.M.; Blanco-Moreno, J.M.; van Logtestijn, R.S.P.; Cornelissen, J.H.C.; Callaghan, T.V. Shrub—tree interactions and environmental changes drive treeline dynamics in the Subarctic. *Oikos* **2012**, *121*, 1680–1690. [CrossRef]

28. Xu, Z.; Hu, T.; Zhang, Y. Effects of experimental warming on phenology, growth and gas exchange of treeline birch (*Betula utilis*) saplings, Eastern Tibetan Plateau, China. *Eur. J. For. Res.* **2012**, *131*, 811–819. [CrossRef]

29. Hartikainen, K.; Kivimäenpää, M.; Nerg, A.M.; Mäenpää, M.; Oksanen, E.; Rousi, M.; Holopainen, T. Elevated temperature and ozone modify structural characteristics of silver birch (*Betula pendula*) leaves. *Tree Physiol.* **2020**, *40*, 467–483. [CrossRef] [PubMed]

30. Riikonen, J.; Lindsberg, M.M.; Holopainen, T.; Oksanen, E.; Lappi, J.; Peltonen, P.; Vapaavuori, E. Silver birch and climate change: Variable growth and carbon allocation responses to elevated concentrations of carbon dioxide and ozone. *Tree Physiol.* **2004**, *24*, 1227–1237. [CrossRef]

31. Riikonen, J.; Percy, K.E.; Kivimäenpää, M.; Kubiske, M.E.; Nelson, N.D.; Vapaavuori, E.; Karnosky, D.F. Leaf size and surface characteristics of *Betula papyrifera* exposed to elevated CO_2 and O_3. *Environ. Poll.* **2010**, *158*, 1029–1035. [CrossRef]

32. Llorens, L.; Penuelas, J.; Emmett, B. Developmental instability and gas exchange responses of a heathland shrub to experimental drought and warming. *Intern. J. Plant Sci.* **2002**, *163*, 959–967. [CrossRef]

33. Cornelissen, T.; Stiling, P.; Drake, B. Elevated CO_2 decreases leaf fluctuating asymmetry and herbivory by leaf miners on two oak species. *Glob. Chang. Biol.* **2003**, *10*, 27–36. [CrossRef]

34. Kaligarič, M.; Tognetti, R.; Janžekovič, F.; Raschi, A. Leaf fluctuating asymmetry of *Myrtus communis* L., affected by increases in atmospheric CO_2 concentration: Evidence from a natural CO_2 spring. *Pol. J. Environ. Stud.* **2008**, *17*, 503–508.

35. Weih, M.; Karlsson, P.S. Growth response of mountain birch to air and soil temperature: Is increasing leaf-nitrogen content an acclimation to lower air temperature? *New Phytol.* **2001**, *150*, 147–155. [CrossRef]

36. Wiegolaski, F.E. *Plant Ecology, Herbivory, and Human Impact in Nordic Mountain Birch Forests*; Springer: Berlin, Germany, 2005.

37. Kozlov, M.V.; Zverev, V. Temperature and herbivory, but not pollution, affect fluctuating asymmetry of mountain birch leaves: Results of 25-year monitoring around the copper-nickel smelter in Monchegorsk, northwestern Russia. *Sci. Total Environ.* **2018**, *640–641*, 678–687. [CrossRef] [PubMed]

38. Lister, A.A. Climate Change Research Group. Natural history collections as sources of long-term datasets. *Trends Ecol. Evol.* **2011**, *26*, 153–154. [CrossRef]

39. Kozlov, M.V.; Cornelissen, T.; Gavrikov, D.E.; Kunavin, M.A.; Lama, A.D.; Milligan, J.R.; Zverev, V.; Zvereva, E.L. Reproducibility of fluctuating asymmetry measurements in plants: Sources of variation and implications for study design. *Ecol. Indic.* **2017**, *73*, 733–740. [CrossRef]

40. Palmer, A.R.; Strobeck, C. Fluctuating asymmetry analyses revisited. In *Developmental Instability: Causes and Consequences*; Polak, M., Ed.; Oxford University Press: Oxford, UK, 2003; pp. 279–319.

41. Cornelissen, T.; Stiling, P. Small variations over large scales: Fluctuating asymmetry over the range of two oak species. *Intern. J. Plant Sci.* **2010**, *171*, 303–309. [CrossRef]

42. SAS Institute. *SAS/Stat. User's Guide, Version 9.2*; SAS Institute: Cary, NC, USA, 2009.

43. Kenward, M.G.; Roger, J.H. An improved approximation to the precision of fixed effects from restricted maximum likelihood. *Computat. Stat. Data Anal.* **2009**, *53*, 2583–2595. [CrossRef]

44. Littell, R.C.; Milliken, G.A.; Stroup, W.W.; Wolfinger, R.D.; Schabenberger, O. *SAS for Mixed Models*, 2nd ed.; SAS Institute: Cary, NC, USA, 2006.

45. Högberg, P.; Näsholm, T.; Franklin, O.; Högberg, M.N. Tamm review: On the nature of the nitrogen limitation to plant growth in Fennoscandian boreal forests. *Forest Ecol. Manag.* **2017**, *403*, 161–185. [CrossRef]

46. Paakkonen, E.; Holopainen, T. 1995. Influence of nitrogen supply on the response of clones of birch (*Betula pendula* Roth) to ozone. *New Phytol.* **1995**, *129*, 595–603. [CrossRef]

47. Engardt, M.; Simpson, D.; Schwikowski, M.; Granat, L. Deposition of sulphur and nitrogen in Europe 1900–2050. Model calculations and comparison to historical observations. *Tellus B Chem. Phys. Meteorol.* **2017**, *69*, 1328945. [CrossRef]

48. Zverev, V.E. Impact of Industrial Contamination on Ecology of Mountain Birch (*Betula pubescens* ssp. *czerepanovii* (Orlova) Hämet-Ahti) in the Kola Peninsula. Ph.D. Thesis, Institute of Plant and Animal Ecology, Yekaterinburg, Russia, March 2012.

49. Zvereva, E.L.; Hunter, M.D.; Zverev, V.; Kozlov, M.V. Factors affecting population dynamics of leaf beetles in a subarctic region: The interplay between climate warming and pollution decline. *Sci. Total Environ.* **2016**, *566–567*, 1277–1288. [CrossRef] [PubMed]

50. Klingenberg, C.P. Phenotypic plasticity, developmental instability, and robustness: The concepts and how they are connected. *Front. Ecol. Evol.* **2019**, *7*, 56. [CrossRef]

51. Hagen, S.B.; Ims, R.A.; Yoccoz, N.G.; Sørlibråten, O. Fluctuating asymmetry as an indicator of elevation stress and distribution limits in mountain birch (*Betula pubescens*). *Plant Ecol.* **2008**, *195*, 157–163. [CrossRef]

52. Murphy, H.T.; Lovett-Doust, J. 2004. Landscape-level effects on developmental instability: Fluctuating asymmetry across the range of honey locust, *Gleditsia triacanthos* (Fabaceae). *Intern. J. Plant Sci.* **2004**, *165*, 795–803. [CrossRef]

53. Royer, D.L.; Mcelwain, J.C.; Adams, J.M.; Wilf, P. Sensitivity of leaf size and shape to climate within *Acer rubrum* and *Quercus kelloggii*. *New Phytol.* **2008**, *179*, 808–817. [CrossRef]

54. Maya-Garcia, R.; Torres-Miranda, A.; Cuevas-Reyes, P.; Oyama, K. Morphological differentiation among populations of *Quercus elliptica* Nee (Fagaceae) along an environmental gradient in Mexico and Central America. *Bot. Sci.* **2020**, *98*, 50–65. [CrossRef]

55. Waddington, C.H. Canalization of development and the inheritance of acquired characters. *Nature* **1942**, *150*, 563–565. [CrossRef]

56. Zverev, V.; Lama, A.D.; Kozlov, M.V. Fluctuating asymmetry of birch leaves did not increase with pollution and drought stress in a controlled experiment. *Ecol. Indic.* **2018**, *84*, 283–289. [CrossRef]

57. Debat, V. Symmetry is beauty—or is it? Grandeur et decadence de l'asymétrie fluctuante. *Médecines Sci.* **2016**, *32*, 774–780. [CrossRef]

58. Diaz-Gil, C.; Palmer, M.; Catalan, I.A.; Alos, J.; Fuiman, L.A.; Garcia, E.; Gil, M.D.; Grau, A.; Kang, A.; Maneja, R.H.; et al. Otolith fluctuating asymmetry: A misconception of its biological relevance? *ICES J. Marine Sci.* **2015**, *72*, 2079–2089. [CrossRef]

59. Kozlov, M.V.; Zverev, V.; Sandner, T.M. Photosynthetic efficiency is higher in asymmetric leaves than in symmetric leaves of the same plant. *Symmetry* **2019**, *11*, 834. [CrossRef]

60. Cornelissen, T.; Stiling, P. Perfect is best: Low leaf fluctuating asymmetry reduces herbivory by leaf miners. *Oecologia* **2005**, *142*, 46–56. [CrossRef] [PubMed]

61. Kozlov, M.V.; Zverev, V.; Zvereva, E.L. Do defoliating insects distinguish between symmetric and asymmetric leaves within a plant? *Ecol. Entomol.* **2018**, *43*, 656–664. [CrossRef]

62. Grime, J.P. *Plant Strategies and Vegetation Processes*; Wiley: New York, NY, USA, 1979.