

1 **Breath of Green Life: Reduction in plant day and night respiration under elevated CO₂**

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7 Plants constantly respire, even when photosynthesising. Current estimates indicate that half the
8 amount of carbon assimilated via terrestrial vegetation is subsequently lost during respiration
9 (Dusenge et al., 2019). Net primary production (NPP), the total carbon (C) fixed by photosynthesis
10 minus the total fixed C lost by respiration, represents the rate at which energy is stored as biomass
11 by plants and made available to the consumers in an ecosystem.

12 Although NPP may seem a simple parameter to determine, in the real world, the rates of both
13 respiration and photosynthesis are differentially affected by multiple factors including water,
14 temperature, carbon dioxide concentration [CO₂] and nutrients. For example, while photosynthesis
15 is known to be stimulated under elevated [CO₂], termed the 'fertilisation effect', the corresponding
16 impact on respiration is poorly understood. This has implications for global models of carbon
17 balance, carbon use efficiency and ecosystem function under climate change (Atkin et al., 2017;
18 Tcherkez et al., 2017).

19 Another complication in assessing NPP relates to the fact that plant respiration occurs both in the
20 light (R_l) or in the dark (R_d). Whilst light partially inhibits leaf respiration, the contribution of R_l versus
21 R_d to CO₂ evolution varies greatly (Crous et al., 2017; Murchie et al., 2022). This is partly a result of
22 day length and differential day versus night temperature in most environments, as well as
23 physiological status and variations in C and nitrogen (N) metabolism between species (Atkin et al.,
24 2007; Gong et al., 2017). However, the specific response of each to changes in conditions is not fully
25 understood, and conflicting responses of R_l to increasing atmospheric [CO₂] have been found.

26 One potential reason for the inconsistent reports is a result of the difficulties in measuring R_l and R_d.
27 The two most common methods used require manipulation of assimilation rates (A) under low
28 irradiance (i.e. less than 150 μmol m⁻² s⁻¹; termed the Kok method, Figure 1A; Kok, 1949) or under
29 low CO₂ (called the Laisk method, Figure 1B; Laisk, 1977); the latter of which is unsuitable for
30 determining the response of respiration to altered [CO₂]. Previous studies have shown that the Kok
31 effect (referring to the break point, or rapid decrease in CO₂ assimilation around the light
32 compensation point) is not caused exclusively by changes in respiration (Gauthier et al., 2020). The
33 Kok method also assumes a constant Photosystem II efficiency (Φ₂) which often results in a lower
34 estimate of R_l relative to the Laisk method. However the incorporation of chlorophyll fluorescence
35 measurements, proposed by Yin et al. (2011), can account for the decline in PSII electron transport
36 efficiency with increasing light intensity. This adapted methodology is known as the Kok-Phi or Yin
37 method. Similarly, the Kok method also assumes a constant chloroplastic [CO₂] (C_c) throughout
38 measurement, which is thought to cause bias but has not been addressed due to the difficulty of
39 measuring mesophyll conductance (g_m) under low light intensities.

40 In this issue of *Plant Physiology*, Sun et al. (2022) explore the short- and long-term responses of leaf
41 day respiration to ambient (410 ppm) and elevated (820 ppm) [CO₂] in wheat (*Triticum aestivum*)
42 and sunflower (*Helianthus annuus*). Using the Kok method, the Kok-phi method and a modified Kok
43 method (termed by the authors as the Kok-Cc method that accounts for changes in chloroplastic

44 [CO₂]), they identified, on average, an 8.4 % reduction in R_i and a 16% reduction in R_d under growth
45 at elevated [CO₂] (Figure 2). However, the authors did not identify any significant change in the R_i : R_d
46 ratio between each treatment. During a short-term change in [CO₂] during measurement, Sun et al.
47 (2022) found an increase in R_i and the R_i : R_d ratio using the Kok and Kok-Phi method but not the
48 Kok-Cc method, a discrepancy attributed to changes arising from differences in intercellular [CO₂].
49 This indicates a tendency to underestimate R_i and overestimate light inhibition under low light
50 intensities using the Kok and Kok-Phi methods. Incorporating intercellular [CO₂] into the
51 methodology indicates that light inhibition of respiration is approximately 6 ± 4 %, equivalent to 26%
52 of the total Kok effect.

53 Similar to previous results, including those from Free-Air CO₂ Enrichment (FACE) studies (Ainsworth
54 and Long, 2005), Sun et al. (2022) found a reduction in both leaf N and chlorophyll content under
55 elevated [CO₂]. Thus the concurrent reduction in R_i and R_d is linked to changes in N metabolism in
56 leaves. Together this indicates a complex relationship between atmospheric CO₂, C- and N- cycles.
57 Whilst the modified Kok-Cc method presented by Sun et al. (2022) presents a more reliable
58 approach towards the assessment of plant respiration, theoretical difficulties still arise due to
59 measurement under low ambient light intensities. Thus, whilst we are now one step closer to
60 understanding plant function in future environments, further work is needed to determine the
61 response of R_i to changes in irradiance.

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64 **Figure Legends**

65 **Figure 1:** Conceptual overview for the calculation of day respiration (R_i) according to two different
66 methods. **A)** The Kok method (Kok, 1984), where the open and closed symbols indicate the different
67 points used to fit the biphasic relationship between CO₂ assimilation and low light intensity, above
68 and below the light compensation point, and R_d indicates the dark respiration rate. **B)** The Laisk
69 method (Laisk, 1977) where the different coloured symbols indicate measurements under different
70 light intensities. Adapted from Yin et al. (2011). N.B. data shown for illustrative purposes only and do
71 not come from real measurements.

72 **Figure 2:** Processes effecting net primary production during the day and night under ambient and
73 elevated carbon dioxide concentrations [CO₂]. Arrow sizes indicate the relative rate of each process,
74 with both day time (R_i) and night-time (R_d) respiration decreasing under elevated [CO₂].

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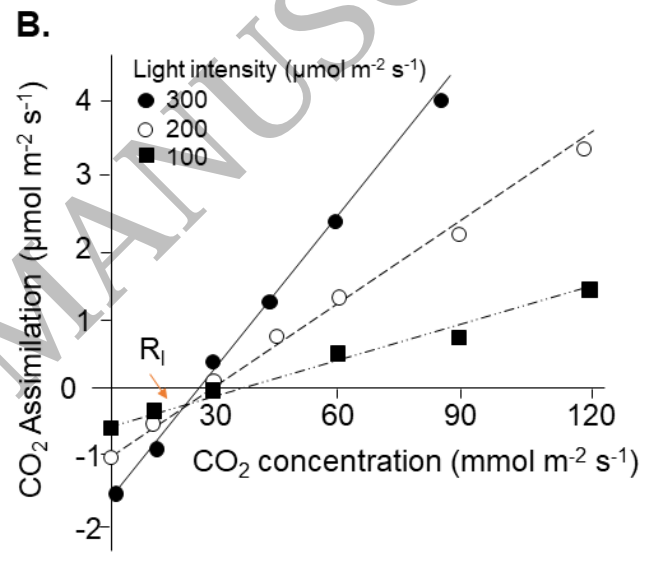
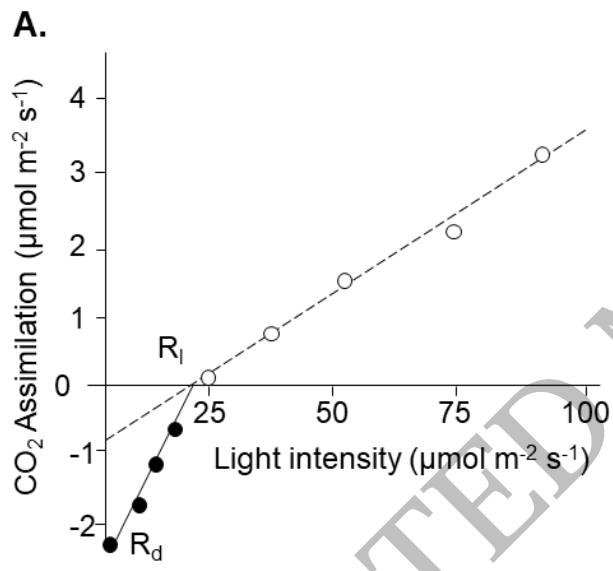
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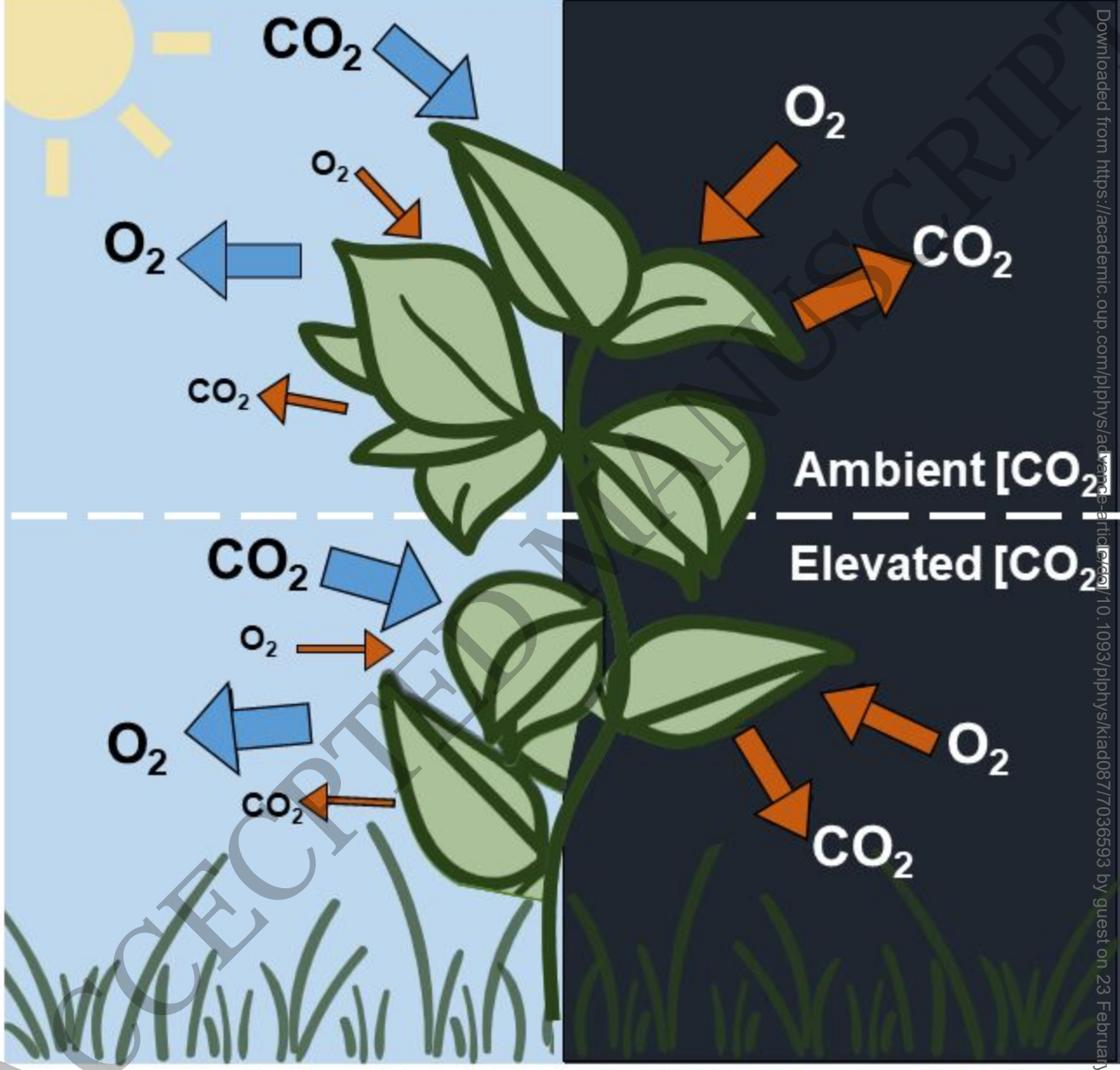
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