Nutrient Supply and Growth Responses of Potato under Elevated CO_2

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Abstract

The global atmospheric CO_2 concentration (ambient CO_2, a[CO_2]) has risen from 280 ppm in pre-industrial times to exceed 400 ppm at present, and it is predicted to continue rising in the future. The elevated CO_2 (e[CO_2]) is expected to enhance crop growth and water-use efficiency (WUE), especially in C_3 species. Potato (*Solanum tuberosum* L.) is the most important non-grain crop in the world and may have great response to e[CO_2] because of its large sink capacity. However, enhancement of crop growth and water economy under e[CO_2] may be dependent on soil nutrient availability. Therefore, it is important to investigate how nutrient supply affect CO_2 effects on plant growth and water use in potato plants under e[CO_2].

Firstly, I quantified potato growth in response to phosphorus (P), nitrogen (N), as well as potassium (K) supply under a[CO_2] and e[CO_2] in growth chambers (GC) to investigate the minimum nutrient and water demands for the maximum plant growth at early growth stage. The e[CO_2] enhanced maximum growth and WUE by 1.5-fold without additional P and water supply. N and water required by potato plants under e[CO_2] was dependent on P supply. Although under P-sufficiency conditions, e[CO_2] increased N but not water demand to obtain maximum growth during the early growth stage, N demand was unchanged and water demand was decreased by e[CO_2] under P-deficiency conditions, probably owing to growth limited by P availability. K supply could remarkably enhance the accumulation of plant biomass under e[CO_2] by promoting tuber formation. Though the maximum plant biomass in response to K supply was not obtained due to narrow range of K supply, CO_2-fertilization effect and WUE were dependent on both P and K supply. Less biomass accumulation in response to K supply in plants with P deficiency, indicates that a balanced nutrient status is crucial for crop production under e[CO_2].

Secondly, to further investigate CO_2 effects on potato plants at different developmental stages and at large scale, I conducted experiments in GC covering the whole growth period of potato plants and in open-top chambers (OTC) at different developmental stages. Total plant and tuber biomass at maturity were increased by 1.4- and 1.6-fold under e[CO_2] in GC when nutrient was sufficient. Enhancement of plant growth under e[CO_2] was smaller in OTC than in GC, though the reason is still unknown. Plant biomass was decreased by e[CO_2] at entire maturity in OTC, which could be due to accelerated senescence under e[CO_2]. Furthermore, the e[CO_2]-mediated senescence could not be prevented, however can be delayed by nutrient supply due to extended growth period, indicating plants with longer lifespan may benefit more from e[CO_2].

Finally, I compared responses of six varieties belonging to various maturing groups to e[CO_2] in order to examine effects of variety earliness on CO_2 effects. It was expected that the variety with longer lifespan can profit more from e[CO_2] during the same growth period. Eventually, the late maturity variety such as Red Moon was found to have greater effect size of e[CO_2], suggesting an importance to maintain vegetative growth under e[CO_2]. Additionally, the varieties (Inkanomezame, Dejima and Red Moon) with higher leaf but lower tuber proportion was found having greater effect sizes of e[CO_2] in total plant biomass comparing to the others. That indicates leaf expansion rather than tuber formation at early growth stage may be more important to fully utilize CO_2 from whole growth period. Lower net assimilation rate (NAR) under e[CO_2] in very early varieties, but higher NAR under e[CO_2] in late varieties indicates the possibility of e[CO_2]-mediated senescence is dependent on earliness of potato plants.

This study suggested that e[CO_2] could remarkably increase maximum plant growth in potatoes without additional P and water demands but with additional N demand at early developmental stage. The enhancement by e[CO_2] could be achieved

even until the end of life cycle unless nutrient is deficient. However, e[CO_2]-induced senescence may cause yield reduction, especially under nutrient deficiency. To against e[CO_2]-induced senescence and fully benefit from e[CO_2], rational fertilization supply and variety breeding are supposed to be deeply considered.

Contents

Abstract I

Abbreviations VII

Chapter 1 1

Nutrient supply and plant responses to elevated CO_2: an overview and research proposal 1

1.1 Climate change of elevated CO_2 and drought 2

1.2 CO_2 effects on crop production and water use 2

1.3 Down-regulation of photosynthesis under elevated CO_2 3

1.4 Effects of nutrient supply on crop growth under elevated CO_2 4

1.5 CO_2 enrichment facilities 8

1.6 Previous research about CO_2 effects on potato growth 8

1.7 Research aims and contents 10

Chapter 2 12

Nutrient and water demands for maximum growth of potato under elevated CO_2 at early growth stage 12

2.0 Introduction 13

Chapter 2.1 16

Trial P 16

2.1.1 Materials and methods 17

2.1.2 Results 20

2.1.3 Discussion 28

2.1.4 Conclusions 32

Chapter 2.2 33

Trial N 33

2.2.1 Materials and Methods 34

2.2.2 Results 38

2.2.3 Discussion 47

2.2.4 Conclusions 52

Chapter 2.3 53

Trial K 53

2.3.1 Materials and methods 54

2.3.2 Results 59

2.3.3 Discussion 66

2.3.4 Conclusions 70

Chapter 3 71

Potato in response to elevated CO_2 under various nutrient status: effects of developmental stages and chambers 71

3.1 Introduction 72

3.2 Materials and methods 73

3.3 Results 78

3.4 Discussion 85

3.5 Conclusions 90

Chapter 4 101

Varietal difference of potato in response to elevated CO_2 101

4.1 Introduction 102

4.2 Materials and methods 104

4.3 Results 107

4.4 Discussion 112

4.5 Conclusions 115

Chapter 5 118

General discussion and conclusions 118

5.1 General discussion 119

5.2 Conclusions and prospect 122

Appendix: description of measurements 123

References 126

Achievements 145

Acknowledgement 147

Abbreviations

(in alphabetical order)

a[CO_2]: ambient CO_2 concentration

DAT: days after transplanting

e[CO_2]: elevated CO_2 concentration

FACE: free-air CO_2 enrichment

GC: growth chambers

g_s: stomatal conductance

HK: high potassium

HN: high nitrogen

HP: high phosphorus

K: potassium

KAE: potassium acquisition efficiency

KUE: potassium utilization efficiency

LAR: leaf area rate

LK: low potassium

LMA: leaf mass per area

LN: low nitrogen

LP: low phosphorus

N: nitrogen

NAE: nitrogen acquisition efficiency

NAR: net assimilation rate

NSC: non-structural carbohydrate

NUE: nitrogen utilization efficiency

OTC: open-top chambers

P: phosphorus

PAE: phosphorus acquisition efficiency

PUE: phosphorus utilization efficiency

RGR: relative growth rate

WUE: water-use efficiency

Chapter 1

Nutrient supply and plant responses to elevated CO_2: an overview and research proposal

1.1 Climate change of elevated CO_2 and drought

The global atmospheric CO_2 concentration has risen from 280 ppm in pre-industrial times to over 400 ppm at present (NOAA, 2021). Moreover, annual growth rate of CO_2 also shows an increasing trend and has reached approximately 2.5 ppm per year at present (NOAA, 2021). According to Fuss et al. (2014), atmospheric CO_2 may become higher than 1000 ppm by 2100 if the increase rate of emissions (2.3% per year) continues.

Increases in ambient CO_2 concentration (a[CO_2]) often cause changes in global environment, as CO_2 is one of the most important drivers of global warming. The rising CO_2 as well as other global warming gases may result in a rise in temperature, and eventually cause drought in the world more seriously (Dai, 2011). The simulated maps for drought status in the United States by Drought Monitor show that area and degree of drought increased greatly whin 11 years from 2010 to 2021 (NOAA, 2021). There is no doubt that crop will grow under elevated CO_2 (e[CO_2]) and suffer more from drought in the future.

1.2 CO_2 effects on crop production and water use

Regardless of global warming, e[CO_2] can efficiently promote crop growth, known as the CO_2-fertilization effect, especially in C_3 plants (Kimball, 1983, 2016; Poorter and Navas, 2003; van der Kooi et al., 2016; Zhang et al., 2021a). This is because CO_2 is the substrate for photosynthesis in plants, and the photosynthetic rate is not yet saturated under current CO_2 conditions, particularly in C_3 plants. Both CO_2 and O_2 compete for the same site on the catalyzing enzyme rubisco, thus photorespiration is suppressed by e[CO_2] in C_3 plants (Drake et al., 1997; Goudriaan et al., 1990; Lemonnier and Ainsworth, 2018); however, C_4 plants are much less affected because photorespiration is already suppressed by a CO_2 concentrating mechanism (Poorter, 1993).

Wittwer (1975) identified water as the second-most limiting factor, behind land area, to increase food production. Terrestrial plants acquire CO_2 and simultaneously lose water via stomata. Stomatal closure increases water-use efficiency (WUE) owing to reduced water loss at the expense of CO_2 acquisition, commonly resulting in growth reduction. However, e[CO_2] actually allows increased WUE without growth reduction, especially in C_3 plants (Brouder and Volenec, 2008). It is anticipated that e[CO_2] can increase WUE by reducing stomatal conductance (g_s) and increasing assimilation rate (Ainsworth and Rogers, 2007; Polley, 2002). Zhang et al. (2021a) reported that photosynthesis was increased approximately 30% by e[CO_2] as an average in all crops examined. Both transpiration and g_s were decreased approximately 20% by e[CO_2], and therefore resulted in an approximately 60% increase in instantaneous WUE. Besides CO_2 effects on plant growth at leaf level, a review study showed an average of 34% reduction in transpiration, 33% increase in yield under e[CO_2] at plant level (Kimball and Idso, 1983).

1.3 Down-regulation of photosynthesis under elevated CO_2

Comparing with rapid responses, long-term (weeks to months) exposure to e[CO_2] often leads to down-regulation of photosynthesis (Ainsworth and Long, 2005; Kramer, 1981; Rogers and Humphries, 2000; Sage, 1994; Stitt and Krapp, 1999). Down-regulation of photosynthesis means a process, in which the initial increase in photosynthesis and growth after transfer to e[CO_2] diminishes over time (Long et al., 2004).

Down-regulation of photosynthesis may be caused by accumulation of non-structural carbohydrate (NSC) (e.g., starch) in source organs due to insufficient sink capacity to use or store NSC (Lemonnier and Ainsworth, 2018; Rey and Jarvis, 1998). Over-accumulation of starch in leaves may damage internal organization of chloroplasts (Pritchard et al., 1997) or hinder CO_2 diffusion in the chloroplasts (Jauregui et al., 2018; Makino and Mae, 1999; Sawada et al., 2001), and then directly lead to the

inhibition of photosynthesis (Ainsworth and Bush, 2011; Stitt, 1991). In addition, the genes required for photosynthesis are repressed by increased hexose sugar (Jang and Sheen, 1994), which may result in an inhibition of photosynthesis under e[CO_2]. However, in some studies, starch and/or sugar accumulation cannot entirely explain down-regulation of photosynthesis under e[CO_2] (Ludewig et al., 1998; Yelle et al., 1989).

Ludewig and Sonnewald (2000) found the e[CO_2]-mediated decline in photosynthesis may be caused by accelerated senescence in tobacco. The e[CO_2] usually causes NSC accumulation and then decreases nitrogen (N) concentration in plant leaves, leading to imbalanced C/N ratio in mature leaves, which is thought to be one of the main factors leading to premature senescence in leaves (Agüera and de la Haba, 2018; de la Mata et al., 2013; Wingler et al., 2004). A high C/N ratio could act as a signal to promote the degradation of photosynthetic product to release N that would be finally allocated to reproductive organs (Havé et al., 2017). Amounts of photosynthetic pigments declined during development of sunflower primary leaves and the decrease was more marked under e[CO_2], suggesting that e[CO_2] accelerates chlorophyll degradation and possibly also leaf senescence (de la Mata et al., 2012, 2013).

Another possible reason for accelerated senescence under e[CO_2] may be related to sugar signaling (Wingler et al., 2006). Pourtau et al. (2006) reported sugar accumulated during senescence with induction of *SAG12*, which is expressed during late senescence. Hexokinse may play critical role in sugar-induced senescence, because comparing with the wild type, hexokinase mutant plants did not accumulate hexoses and senescence was delayed (Pourtau et al., 2006).

1.4 Effects of nutrient supply on crop growth under elevated CO_2

Plant in response to CO_2, has been known to interact with other environmental factors, including soil nutrient availability (Conroy, 1992). Growth promotion by e[CO_2] is

often limited by nutrient deficiency (Campbell and Sage, 2006; Lynch and St. Clair, 2004; Terrer et al., 2019). Moreover, e[CO_2] decreased nutrient concentration in plants, including the three most important nutrients, namely N, phosphorus (P), and potassium (K) (Taub and Wang, 2008). More nutrient supply may be required for crop to reach optimal growth under e[CO_2].

1.4.1 Nitrogen

N is considered the main factor governing plant growth and crop yield (Marschner, 1995). Limited N supply affects both cell metabolism and plant growth by decreasing protein and chlorophyll content (Agüera et al. 2010). Photosynthetic rate is related to leaf N concentrations (Larigauderie et al., 1988) because of the important role of N in the formation of Rubisco protein (Stitt, 1991). N concentration under e[CO_2] is usually lower than that under a[CO_2] (Taub and Wang, 2008), which is partly due to the increase in NSC accumulation. NSC further accumulates in N deficient plants under e[CO_2] (Sims et al., 1998). It has been frequently observed that N deficiency accelerates down-regulation of photosynthesis under e[CO_2] (Pettersson and McDonald, 1994; Stitt and Krapp, 1999), because N deficiency limits growth and activity of sink tissues. In previous studies, crop growth under e[CO_2] conditions was inhibited by N deficiency (Ainsworth and Long, 2005; Kim et al., 2003; Reich et al., 2014; Wong, 1979). Furthermore, low N was reported to accelerate plant senescence that may also be induced by e[CO_2] (Agüera and de la Haba, 2018; Aoyama et al., 2014). Under limited N supply, plants trigger senescence in order to mobilize N through degradation of proteins (Masclaux et al., 2000). Therefore, increasing N supply may be an efficient means to enhance plant growth under e[CO_2] by delaying senescence.

N supply was also reported to improve WUE in various crops, including wheat (Shangguan et al., 2000), maize (Al-Kaisi et al., 2003), safflower (Dordas and Sioulas, 2008), and potato (Badr et al., 2012). However, how N supply affect WUE under e[CO_2] just has been investigated in a very small amount of studies. Franzaring et al. (2011)

and Manderscheid et al. (2018) reported an increase in WUE under e[CO_2] could be further enhanced as N supply increases. N deficiency decreases g_s, and further reduces carbon assimilation rate (Broadley et al., 2001). g_s for water loss and CO_2 acquire is determined primarily by stomata opening and density (Parlange and Waggoner, 1970), which are affected by both genetical and environmental factors (Hetherington and Woodward, 2003; Lake et al., 2002; Woodward et al., 2002). Zhu et al. (2020) reported an increase in g_s with proper N addition in Manchurian ash and Mongolian oak, and an increase in stomatal density and stomatal opening with N addition was also observed. Yan et al. (2012) only observed an increase in stomatal density, however, a reduction in stomata opening with the increases in N supply in potato plants.

1.4.2 Phosphorus

P is an essential macronutrient required for plant growth and metabolism (Marschner, 1995). P deficiency was reported to limit more than 40% crop production of arable land in world (Balemi and Negisho, 2012). Additionally, P deficiency has been reported to cause starch accumulation by affecting the photosynthetic electron transport chain (Carstensen et al., 2018), and starch accumulation involves in down-regulation of photosynthesis under e[CO_2] (Ainsworth and Bush, 2011). Recent review research by Jiang et al. (2020) suggested that low P supply constrains plant responses to e[CO_2], including plant biomass and WUE. P supply remarkably increased crop biomass under e[CO_2] (Seneweera et al., 1997).

That P supply improvs WUE has been reported in many studies (Chaudhary et al., 2018; Farahani et al., 2008; Payne et al., 1992; Sun et al., 2015). Enhancement of WUE by P addition, however depends on various environment conditions, including CO_2 conditions (Conroy et al., 1988; Grünzweig and Körner, 2003). As for the effects of P supply on g_s, stomatal opening and density, inconsistent results have been reported. Singh et al. (2013a, 2013b) found g_s increased with an increase in P supply in cotton, however, stomatal density decreased with the increases in P supply. To the contrary,

Sun et al. (2015) reported a reduction of g_s with P supply in potato. Sekiya and Yano (2008) observed an increase in stomatal density with the increases in P supply in cowpea. Similarly, Sarker et al. (2010) reported higher stomatal density and larger size of stomata with P supply in maize.

1.4.3 Potassium

K is one of the essential macronutrients playing important roles in plant growth and development (Leigh and Wyn Jones 1984; Schachtman and Shin 2007; Wang and Wu 2013). K involves in many physiological processes, including enzyme-activation processes, thus, K deficiency can limit the formation of starch (Nitsos and Evans 1969), impair ATP formation and phloem loading of carbohydrates, and increase the plant respiration (Römheld and Kirkby 2010). K is crucial for maintaining photosynthesis by facilitating CO_2 diffusion through the leaf mesophyll (Jákli et al., 2017; Singh and Reddy, 2018; Tränkner et al., 2018). It also plays important roles in transport of solutes through the phloem including sucrose movement from shoot to root and to the sink tissues such as fruits (Cakmak et al., 1994; Hayashi and Chino 1990). Consequently, K deficiency leads to the accumulation of sucrose in source leaves and restricts sink strength. It has been generally suggested that plants with larger sink strength could benefit more from e[CO_2] (Marschner, 1995). Hence, plant growth under e[CO_2] may be greatly inhibited under K deficiency, as reported in soybean (Singh and Reddy, 2017).

WUE was enhanced by 30% under adequate K supply at plant level in wheat, though no effect was observed at leaf level (Jákli et al., 2016). Enhanced WUE by K supply has also been reported in other species, such as barley (Andersen et al., 1992), sunflower (Fournier et al., 2005; Jákli et al., 2017), and olive tree (Arquero et al., 2006). K is an important factor adjusting stomatal movement; severe K deficiency decreased g_s under both a[CO_2] and e[CO_2] in soybean (Singh and Reddy, 2017). However, g_s increased with K starvation in olive tree (Arquero et al., 2006). Stomatal opening is

associated with K concentration due to its role in osmotic pressure (Humble and Raschke, 1971). Stomatal density was increased by K deficiency in previous studies (Nagarajah and Ratnasuriya, 1978; Turcios et al., 2021; Zhang et al., 2021b). However, the interaction between K and CO_2 on water use is unclear.

1.5 CO_2 enrichment facilities

There are several CO_2 enrichment facilities utilized to study CO_2 effects on crop growth at different scales. The most applied CO_2 enrichment facilities are growth chambers (GC), open-top chambers (OTC), and free-air CO_2 enrichment (FACE). FACE is in field and under natural environment, however, it is hard to applied because of high cost. Most studies on CO_2 effects have been conducted in GC due to its feasibility for controlling growth conditions with low cost. OTC allows experiments under natural sunlight and irradiance with lower cost for CO_2 control than FACE. A review study showed that the overall CO_2 effect on crop biomass was little affected by CO_2 enrichment facilities, however variance differed between experimental setups (van der Kooi et al., 2016). Long et al. (2006) argued that crop yield increases at e[CO_2] were less in FACE than in enclosure studies. Dale (1982) pointed that reduced irradiance or the alteration of the natural light spectrum can modify plant development and override any effect of other variables. However, a study for soybeans and winter wheat grown in FACE and OTC systems at the same time and location found that increase in yield was much higher in OTC than FACE even though irradiance and light spectrum were thought similar between the two CO_2 enrichment facilities (Bunce, 2016). In another study, no significant difference on relative growth response to e[CO_2] between OTC and FACE (Kimball et al., 1997). It is still unclear what caused the difference in CO_2 effects on crop growth between different CO_2 facilities.

1.6 Previous research about CO_2 effects on potato growth

Potato (*Solanum tuberosum* L.) is the most important non-grain crop in the world (Raymundo et al., 2018). It ranks as the fourth largest global food crop only after maize,

rice, and wheat (http://faostat.fao.org). Similar to other crops with the C_3 biochemical pathway, positive responses of potato to e[CO_2] have been reported (Finnan et al., 2005). Potato may have great potential for increasing growth under e[CO_2] because of its large below-ground sink capacity for carbon, which has generally been proposed as a critical trait for optimizing plant production under e[CO_2] (Marschner, 1995).

The first article on the fertilizing effect of e[CO_2] was reported by Gradewitz (1920), where production was increased by 2.75- and 1.70-fold in tomatoes and cucumbers, respectively. Research focusing on CO_2 effects has been reported in many plant species, such as rice (Hasegawa et al., 2013), wheat (Högy et al., 2013), soybean (Ainsworth et al., 2002), and potato (Miglietta et al., 1998). The research about CO_2 effects on potato was firstly conducted in GC by researchers from the US in 1977 (Ku et al., 1977), and then studies in FACE have been started from 1997 in Italy (Miglietta et al., 1997). Later, the Commission of European Union funded a research named 'CHanging climate and potential Impacts on Potato yield and quality project (CHIP)' from 1998 to 2000 (de Temmerman et al., 2000). This project allowed European network on potato in response to increasing CO_2 and ozone under both OTC and FACE. From 2008, studies on potato in soil-plant-atmosphere research (SPAR) have been started by Fleisher et al. (2008a, 2008b). In these previous studies, responses of potato to e[CO_2] have been investigated, interacting with ozone (Bindi et al., 2002; Craigon et al., 2002; Fangmeier et al., 2002; Pleijel et al., 2002; Vorne et al., 2002), irradiance (Cao et al., 1994; Nitithamyong et al., 1999; Piao et al., 2004; Wheeleret al., 1989), temperature (Cao et al., 1994; Nitithamyong et al., 1999), or drought (Barnaby et al., 2015, 2019; Fleisher et al., 2008a, 2008b, 2013a, 2014; Yang et al., 2015). However, how nutrient supply affects CO_2 effects on potato plants have been little examined except for Fleisher et al. (2012, 2013b). In this study, I focused on growth responses of potato plants to e[CO_2] under various nutrient status, thus to provide some valuable data about potato production in an increasing CO_2 environment.

1.7 Research aims and contents

Nutrient, as an important constraint for potato production under e[CO_2], has been little examined with the interaction of CO_2. This study aimed to investigate the interaction between CO_2 and nutrient supply, including N, P, K, on potato growth at controlled-environment conditions as well as open-air conditions. The whole thesis is composed of the following five chapters.

In Chapter 1, I made a brief introduction about the CO_2 effects on plant growth. The e[CO_2] is expected to enhance plant growth, however, the increment may be dependent on soil nutrient availability. How nutrient supply affects CO_2 effects on potato growth has been little examined. Therefore, my research topic is nutrient supply and growth responses of potato under e[CO_2].

In Chapter 2, I set five or six nutrient supply rates to get the response curve of potato plants under e[CO_2]. An adequate and balanced nutrient supply is important for achieving a high yield in potato plants under e[CO_2], but excessive nutrient supply will cause a waste of fertilizes and even environmental pollution. Therefore, I quantified the minimum nutrient demand for the maximum growth in potato plants under a doubling CO_2 condition in GC at relatively early developmental stage and small scale. Furthermore, I also investigate water demand for the maximum growth under comparative CO_2 conditions, as water use for crop production is also important, especially in arid areas.

In Chapter 3, I compared responses of the same potato cultivar to e[CO_2] in different CO_2 enrichment facilities (GC and OTC) and at different developmental stages under various nutrient supply to verify CO_2 effects until maturity and at a relatively large scale. Since experiments under natural and fully open-air conditions are preferable for the research on agricultural production. Thus, it is necessary to verify CO_2 effects in long-term exposure to e[CO_2] and at large scale.

In Chapter 4, I compared responses of six potato varieties belonging to different maturing groups to e[CO_2] to find out the preferable potato variety under e[CO_2]. Since CO_2 effects also varies within the same species (Schapendonk et al., 2000), it is important to find the preferable varieties of potato under e[CO_2] and what characters contribute to the superiority under e[CO_2].

In Chapter 5, I made a general discussion and conclusion about this study.

Chapter 2

Nutrient and water demands for maximum growth of potato under elevated CO_2 at early growth stage

2.0 Introduction

Potato is the most important non-grain crop in the world (Raymundo et al., 2018). In a study on five species, down-regulation of photosynthesis was observed in four of the species studied, but not in potato (Sage et al., 1989), suggesting that the large sink capacity of potato tubers may entail a high potential for potato yield enhancement under e[CO_2].

A critical factor that may limit plant production under e[CO_2] is soil nutrient availability (Lenka and Lal, 2012; Rogers et al., 1999). Potato production is fertilizer intensive, requiring relatively large amounts of nutrients including P (Alvarez-Sánchez et al., 1999; Singh, 1987), N (Ainsworth and Long, 2005; Reich et al., 2014), and K (Westermann, 2005; Witold et al., 2017). Although there are numerous studies on potato plants in response to P (Alvarez-Sánchez et al., 1999; Fleisher et al., 2012, 2013b), N (Mokrani et al., 2018; Vos, 1997; Vos and van der Putten, 1998), and K (Koch et al., 2019; Li et al., 2015), only a few studies considered the possible interaction of CO_2 and nutrient supply on potato growth (Fleisher et al., 2012, 2013b). How e[CO_2] affects the nutrient requirement for the maximum growth in potato plants is still unclear.

Plant nutrition status is a direct indicator for evaluating nutrient and fertilizer management. One of such indices is critical nutrient concentration, defined as the minimum nutrient concentration the crop requires to reach 90% of maximum growth (Chisholm et al., 1981; Conroy, 1992). Critical P concentration (critical [P]) reportedly became higher, however critical N concentration (critical [N]) became lower under e[CO_2] in cotton and wheat (Rogers et al., 1993). It has not yet been examined if critical nutrient concentration is affected by e[CO_2] in potato plants. Although Fleisher et al. (2013b) showed that P supply requirement for growth was unchanged by e[CO_2] in potato plants, critical [P] was not investigated in their research. Hence, more rigorous and quantitative data covering the range from nutrient-deficiency to nutrient-

sufficiency as growth rate becomes saturated, are needed to further assess e[CO_2] effects and to develop appropriate nutrient management in potato cropping.

Another important factor which may affect crop production under e[CO_2] is water. The e[CO_2] allows increased WUE without growth reduction, especially in C_3 plants (Brouder and Volenec, 2008). An important question is how e[CO_2] affects water demand of crop plants, as approximately 70% of the fresh water consumed globally is used in agriculture (Clarke and King, 2004). Interestingly, it has been anticipated that e[CO_2] will increase WUE by decreasing g_s and increasing assimilation rate (Ainsworth and Rogers, 2007; Polley, 2002). In addition, WUE can be enhanced by nutrient supply, including P (Farahani et al., 2008; Sun et al., 2015), N (Brueck, 2008), and K (Andersen et al., 1992; Fournier et al., 2005; Jákli et al., 2017). How nutrient supply affects WUE under e[CO_2] in potato plants remains unclear. To address the above questions, it is necessary to measure daily water consumption by the plant under different nutrient supply and CO_2 conditions, and then estimate productive WUE as biomass increase per unit water used (i.e. accumulated daily water consumption) at the individual plant level.

In this chapter, three independent experiments were conducted, designated as Trial P, Trial N, and Trial K, to investigate potato response to P, N, and K respectively. The aim was to quantify the growth response of the potato plant to nutrient supply under two different CO_2 conditions to know how much nutrient and water are required for maximum growth under each CO_2 condition. As phosphate rock, from which phosphate fertilizers are made, is a finite and non-renewable resource that may be exhausted in the near future (Vaccari, 2009), crops may be grown under e[CO_2] and P-deficient condition. Therefore, how P nutrition affects N and K demands under e[CO_2] in potato plants was also investigated in Trials N and K, respectively.

In Trial P in Chapter 2.1, I quantified the growth response of potato plant to six P supply rates under two CO_2 conditions. The following three questions were addressed: 1) to what extent can maximum biomass accumulation be enhanced by e[CO_2] in potato

plants?, 2) how much P is required to achieve maximum biomass accumulation and, will e[CO_2] increase the plant P requirement?, and finally 3) how does e[CO_2] affect water consumption by the plant to reach maximum biomass accumulation under varying P supply?

In Trial N in Chapter 2.2, I quantified the growth response of potato plant to five N supply rates under different CO_2 conditions and P supply. The following questions were addressed: 1) how much N is required to achieve maximum biomass accumulation in potato plants, and whether it is altered by e[CO_2] and P nutrition?, and 2) how e[CO_2] and P nutrition affect water consumption by potato plants to reach maximum biomass under varying N supply rates?

In Trial K in Chapter 2.3, I examined growth response of potato plants to e[CO_2] under different K and P supply rates. The following questions were addressed: 1) how K supply affects biomass accumulation under e[CO_2]?, 2) how P-K balance affects CO_2-fertilization effect?, and 3) how e[CO_2] affects WUE under varied P and K nutrition statuses.

Chapter 2.1

Trial P

Quantifying phosphorus and water demand to attain maximum growth of *Solanum tuberosum* in a CO_2-enriched environment

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2.1.1 Materials and methods

Experimental design and growing conditions

A pot experiment was undertaken in GC (LPH-410 SPC, Nippon Medical & Chemical Instruments Co. Ltd, Japan). The environmental conditions inside the chambers were set as follows: light intensity, 400 µmol m^{-2} s^{-1}; relative humidity, 60%; temperature, 25 and 17 °C, light and dark, respectively; and photoperiod, 14 and 10 h, light and dark, respectively. Relative humidity was not successfully controlled due to malfunction of the chambers; it was approximately 36% and 48%, light and dark, respectively. The CO_2 concentrations were controlled at approximately 400 ppm for a[CO_2] and 800 ppm for e[CO_2]. The plants and CO_2 concentrations were switched weekly between the two chambers to minimize any potential chamber effects. A CO_2 recorder (TR-76Ui, T&D Inc, Japan) was placed inside each chamber to monitor practical conditions in the chambers every 10 min (Figure 2.1.1).

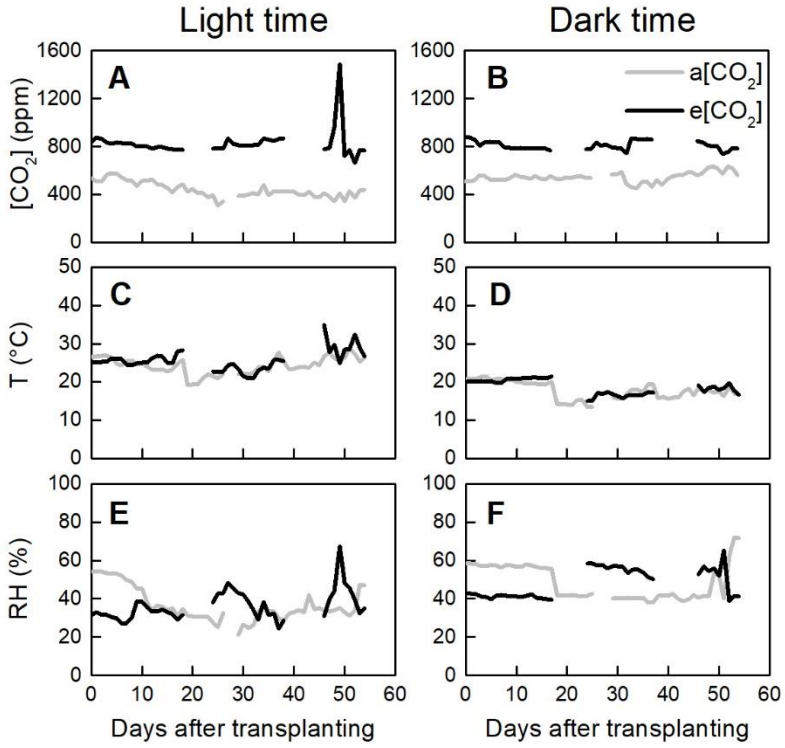

Figure 2.1.1 Actual conditions in growth chambers during growth period (54 days after transplanting). Actual CO_2 concentrations at light time **(A)** and dark time **(B)**. Actual temperature (T) at light time **(C)** and dark time **(D)**. Actual relative humidity (RH) at light time **(E)** and dark time **(F)**.

Naturally sprouted potato tubers (cv. 'Irish Cobbler') were transplanted into 1-L pots (diameter, 11.3 cm; depth, 14 cm; one plant per pot) filled with 580 g of dry andosol. Before transplanting, N (0.4 g N kg^{-1} dry soil) and K (0.4 g K_2O kg^{-1} dry soil) were mixed with the soil in the form of ammonium sulphate (21.0% N) and potassium chloride (60.0% K_2O), respectively. At 24 and 40 days after transplanting (DAT), respectively, 0.77 g KNO_3 dissolved in tap water was added to each pot to provide enough N and K for plant growth. Calcium superphosphate (17.5% P_2O_5) was uniformly mixed with the soil to control P levels at 0, 0.4, 0.8, 1.2, 2.4, or 3.6 g P kg^{-1} of dry soil (hereafter, these treatments are designated as P0, P0.4, P0.8, P1.2, P2.4, and P3.6, respectively). Soil water condition was kept at 60% (w/w) by adding tap water to each pot to compensate for water-loss due to transpiration until 40 DAT, and then kept at 80% (w/w) until harvest to avoid drought-like conditions due to rapid daily soil water consumption, which is accompanied by growth. The experiment was organized following a factorial design (two CO_2 concentrations × six P supply rates) with six biological replications.

Harvest and sampling

All plants were harvested on the 54 DAT. Before harvest, three young fully expanded leaves were sampled from each plant to ensure sample size was sufficient for starch and P quantification. Sampled leaves were immediately frozen in liquid N and then dried for starch and P analysis. At harvest, the remaining leaves, stems, roots, and tubers were separated and dried in an oven at 80 °C to constant mass for dry weight determination. All samples were then ground to powder for P quantification. Soil samples were collected after harvesting and then dried at 80 °C to constant mass for available P and pH analysis. Leaf area and root morphological parameters (root length and root surface area) were analyzed in a flatbed scanner (EPSON EXPRESSION 10000XL, Seiko Epson Co, Japan) using software WinRHIZO Pro LA2400 (Regent Instruments Inc, Canada) before drying.

Stomatal conductance and stomatal density

On harvest day, g_s of the youngest fully expanded leaf was measured on the adaxial surface at 8:00-12:00 in the morning with a leaf porometer (SC-1, Decagon Devices Inc, USA). Immediately after measurement of g_s, the same leaves were coated with nail polish; next, imprints were taken from each leaf and mounted on a glass microscope slide to count the number of stomata under the microscope (SZ61, OLYMPUS Co, Tokyo, Japan). Five observations of each imprint were randomly selected to count the number of stomata; thus, data presented are means of five individual measurements per leaf.

Statistical analysis

The experiment was organized following a factorial design with two CO_2 concentrations and six P supply rates with six replications (except for the e[CO_2] P3.6 treatment, as one plant in this treatment died after transplanting), data are expressed as mean ± standard error (S.E.) for six (or five) biological replicates. Data were analyzed in SPSS 16.0 (SPSS Inc., Chicago, IL, USA) using two-way analysis of variance (ANOVA) at the 0.05 probability level. Simple regressions were analyzed in Origin 9.0 (https://www.originlab.com). Coefficients of the exponential equations ($y = a - b \times c^x$) from regression relationships between foliar [P] and total biomass were used to calculate critical [P] ($\text{Critical [P]} = \text{Log}(a/10b, c)$). Foliar [P] was defined as the [P] of all leaves, including young leaves and remaining leaves. P acquisition efficiency (PAE) and P utilization efficiency (PUE) were calculated after Boucho et al. (2019),

PAE (%) = (P content in P-treated plant – P content in P0 plant) × 100% (g)/ P supply (g);

PUE (g mg^{-1}) = total plant biomass (g)/ total plant P content (mg).

2.1.2 Results

Plant growth and biomass partitioning

Based on plant appearance, soil P availability obviously limited growth below P1.2 under a[CO_2], while at P0.8 growth seemed to be similar to that at P1.2 under e[CO_2] (Figure 2.1.2).

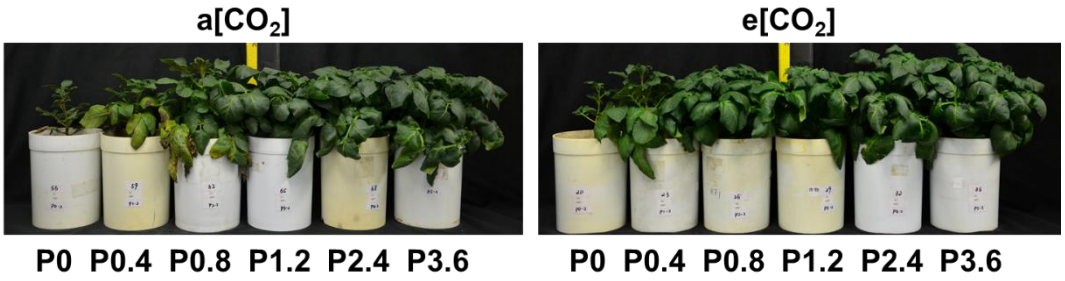

Figure 2.1.2 Appearance of potato plants at harvest (54 days after transplanting).

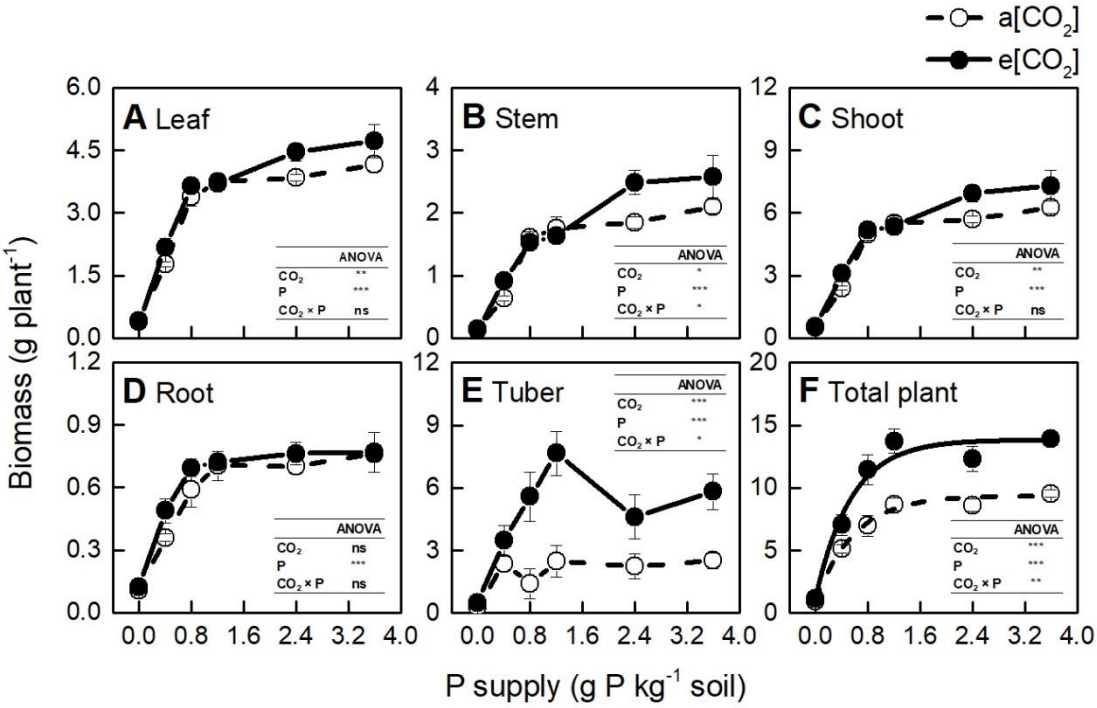

Figure 2.1.3 Biomass of several organs of potato plants grown under a[CO_2] (439 ± 9 ppm) and e[CO_2] (825 ± 17 ppm) with different P supply rates (0, 0.4, 0.8, 1.2, 2.4, and 3.6 g P kg^{-1} soil). Data in each plot are means ± S.E. (n = 6 or 5 biological replicates for each treatment). Statistical comparisons (two-way ANOVA) between CO_2 concentrations and P supply rates as well as their interaction (CO_2 × P) are presented. **(A)** Leaf biomass; **(B)** stem biomass; **(C)** shoot biomass; **(D)** root biomass; **(E)** tuber biomass; and **(F)** total plant biomass. Regressions in (F) are as follows: a[CO_2]: $y = 9.378 - 8.403 \times 0.179^x$, R^2 = 0.996, P < 0.001; e[CO_2]: $y = 13.808 - 12.670 \times 0.156^x$, R^2 = 0.992, P < 0.001.

Plants were harvested on the 54 DAT. In terms of dry weight, e[CO_2] significantly increased leaf, stem, and tuber, but not root biomass (Figure 2.1.3). As for P rates, total biomass increased with increasing P supply rates and reached a maximum at 1.2 g P kg^{-1} soil under both CO_2 conditions tested (Figure 2.1.3F). There was a significant effect from the interaction between CO_2 and P supply on total plant biomass (P = 0.006), tuber biomass (P = 0.012), and stem biomass (P = 0.022). Maximum plant total biomass was enhanced by 47% under e[CO_2] compared to a[CO_2]; however, e[CO_2] did not alter P supply requirement for total plant maximum biomass (Figure 2.1.3F).

Dry matter assimilation and partitioning are important processes determining crop productivity. Our study suggested that e[CO_2] enhanced tuber growth (Figure 2.1.4). Thus, at harvest, the dry matter proportion in the leaf (44 ± 1.5%) was highest under a[CO_2]. However, a greater proportion of biomass was allocated to tubers (44 ± 2.8%) under e[CO_2] at the expense of dry matter accumulation in stems, roots, and leaves. As for P effects, the largest P rates reduced the proportion of dry matter allocated to tubers at P0.8 and P2.4 under a[CO_2] and e[CO_2], respectively, likely due to shoot overgrowth induced by high P supply.

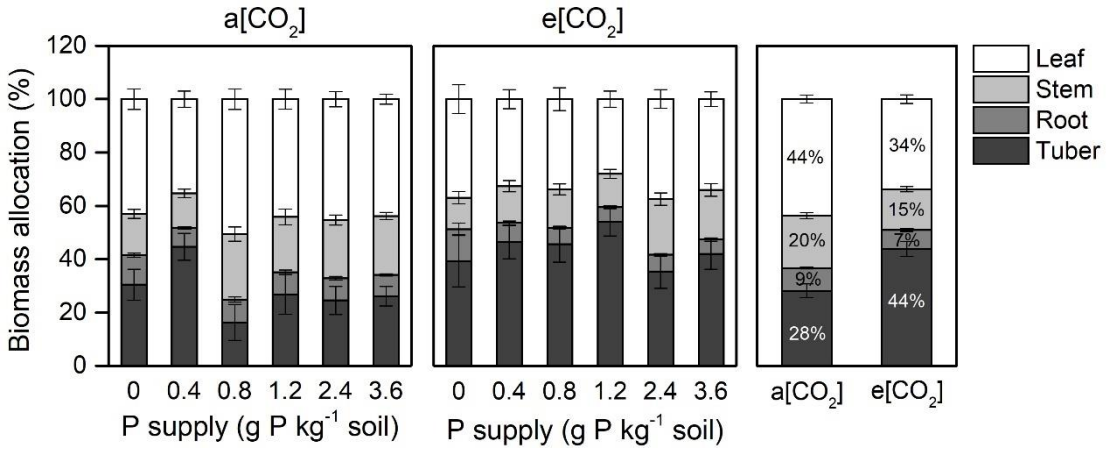

Figure 2.1.4 Biomass partitioning in organs of potato plants grown under a[CO_2] (439 ± 9 ppm) and e[CO_2] (825 ± 17 ppm) with different P supply rates (0, 0.4, 0.8, 1.2, 2.4, and 3.6 g P kg^{-1} soil). Data in each plot are means ± S.E. (n = 6 or 5 biological replicates for each treatment).

On the other hand, e[CO_2] and P deficiency increased starch accumulation in young leaves (Figure 2.1.5A), which could further reduce CO_2 assimilation (Nakano et al., 2000); indeed, P deficiency has been reported to cause starch accumulation by affecting photosynthetic electron transport chain (Carstensen et al., 2018). This study showed that starch concentration correlated negatively with P concentration in young leaves, regardless of CO_2 conditions (Figure 2.1.5B); this finding suggested that increased starch accumulation under e[CO_2], especially at higher P supply rates, could be related to reduced P concentration.

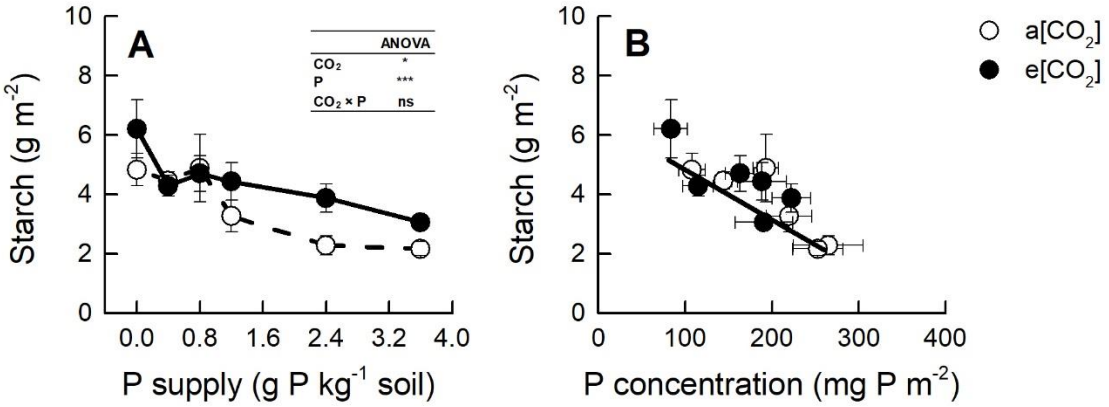

Figure 2.1.5 (A) Starch concentration of the young fully expanded leaves in potato plants grown under a[CO_2] (439 ± 9 ppm) and e[CO_2] (825 ± 17 ppm) with different P supply rates (0, 0.4, 0.8, 1.2, 2.4, and 3.6 g P kg^{-1} soil). Data in each plot are means ± S.E. (n = 6 or 5 biological replicates for each treatment). Statistical comparisons (two-way ANOVA) between CO_2 concentrations and P supply rates as well as their interaction (CO_2 × P) are presented. **(B)** Relation between starch concentration and P concentration of the young fully expanded under a[CO_2] and e[CO_2]. Regression is as follows: y = -0.017x + 6.49, R^2 = 0.724, P < 0.001.

Water use and water-use efficiency

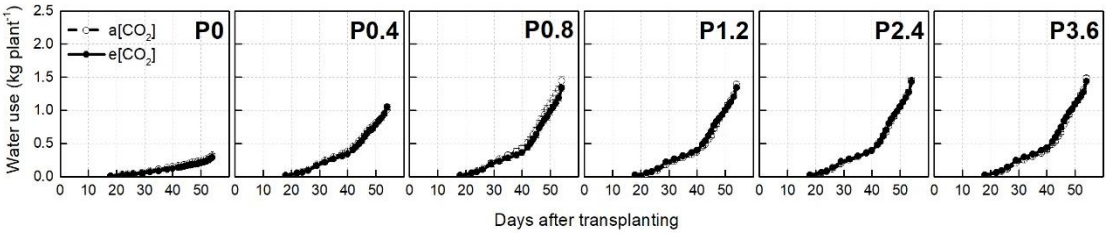

Figure 2.1.6 Water use by potato plants over the experimental period (54 days after transplanting) under a[CO_2] (439 ± 9 ppm) and e[CO_2] (825 ± 17 ppm) with different P supply rates (0, 0.4, 0.8, 1.2, 2.4, and 3.6 g P kg^{-1} soil). Data in each plot are means ± S.E. (n = 6 or 5 biological replicates for each treatment).

I monitored time-course changes in cumulative transpiration as water use in potato plants (Figure 2.1.6).

Water use was unaffected by e[CO_2] at each P level (Figure 2.1.7A). The significant increase in WUE under e[CO_2] might be attributed to biomass increase (Figures 2.1.3 and 2.1.7). Water use increased with increasing P supply rate and peaked at P0.8 under both CO_2 conditions tested. Similarly, WUE was enhanced by increasing P supply rate and reached its maximum at P1.2 under both CO_2 conditions tested. The two-way ANOVA showed that there was a significant interaction (P = 0.010) between CO_2 and P supply rates on WUE, indicating that WUE increased with e[CO_2] in a P supply-dependent manner.

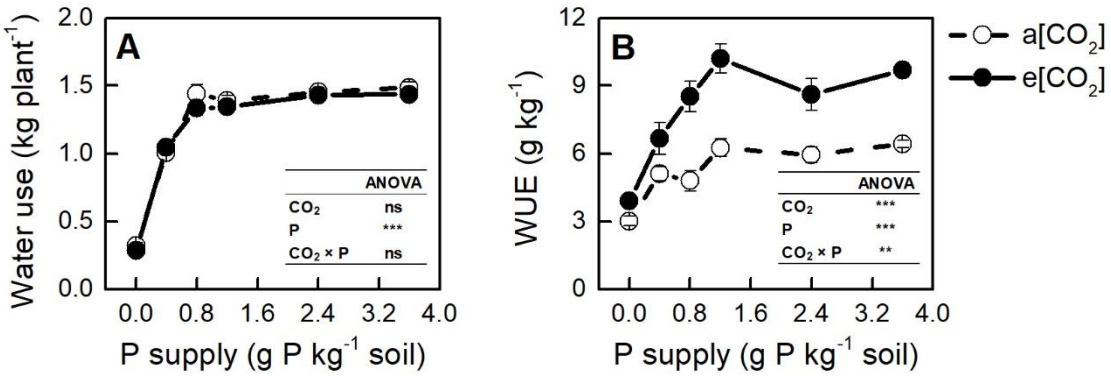

Figure 2.1.7 (A) Water use and **(B)** water-use efficiency (WUE) of potato plants grown under a[CO_2] (439 ± 9 ppm) and e[CO_2] (825 ± 17 ppm) with different P supply rates (0, 0.4, 0.8, 1.2, 2.4, and 3.6 g P kg^{-1} soil). Data in each plot are means ± S.E. (n = 6 or 5 biological replicates for each treatment). Statistical comparisons (two-way ANOVA) between CO_2 concentrations and P supply rates as well as their interaction (CO_2 × P) are presented.

Leaf area was slightly larger in e[CO_2] than in a[CO_2] at 0.4, 0.8, and 2.4 P supply levels and similar among the remaining treatments (Figure 2.1.8A) while P levels had a major effect on this parameter. Therefore, unchanged water use by e[CO_2] could be related to stomatal density or g_s. As for stomatal density, there was no significant difference between a[CO_2] and e[CO_2], but it increased with increasing P supply (Figure 2.1.8B). As Figure 2.1.8C shows, e[CO_2] largely decreased g_s compared to a[CO_2]; further, g_s decreased with increasing P supply and reached a minimum at P1.2

under both CO_2 conditions. Therefore, increased WUE under e[CO_2] was likely related to reduced g_s , which was also affected by P supply level.

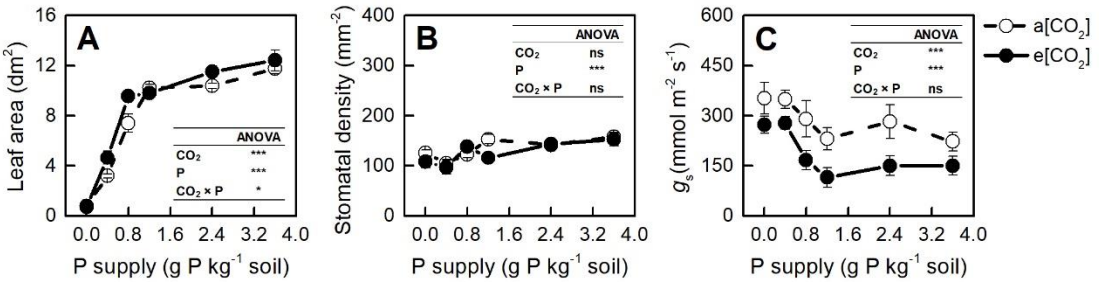

Figure 2.1.8 (A) Leaf area, **(B)** stomatal density, and **(C)** stomatal conductance (g_s) of potato plants grown under a[CO_2] (439 ± 9 ppm) and e[CO_2] (825 ± 17 ppm) with different P supply rates (0, 0.4, 0.8, 1.2, 2.4, and 3.6 g P kg^{-1} soil). Data in each plot are means ± S.E. (n = 6 or 5 biological replicates for each treatment). Statistical comparisons (two-way ANOVA) between CO_2 concentrations and P supply rates as well as their interaction (CO_2 × P) are presented.

Critical phosphorus concentration

I assessed critical [P] in potato plants because P demand by plant under e[CO_2] is likely to increase due to the stimulation of photosynthesis (Jin et al., 2015). In the present study, critical [P] was similar under a[CO_2] (117 mg P m^{-2}) and e[CO_2] (104 mg P m^{-2}) (Figure 2.1.9).

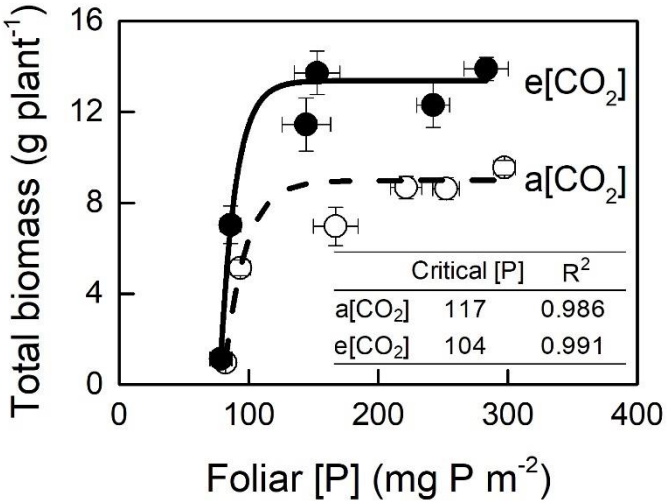

Figure 2.1.9 Relations between total biomass with foliar P concentration (Foliar [P]). Critical [P] is defined as the minimum concentration of P required by the crop to reach 90% of maximum growth. Critical [P] and R^2 values for regressions are presented. Data in each plot are means ± S.E. (n = 6 or 5 biological replicates for each treatment). Regressions are as follows: a[CO_2]: $y = 8.985 - 1392.014 \times 0.939x$, R^2 = 0.986, P < 0.001; e[CO_2]: $y = 13.375 - 9835.24 \times 0.918x$, R^2 = 0.991, P < 0.001.

[P] in all plant organs varied with CO_2 conditions and P supply rates tested; further, two-way ANOVA results confirmed a strong interaction between these two factors (Figure 2.1.10). Additionally, [P] decreased in all plant organs and at the whole plant level under e[CO_2], compared with a[CO_2], but it was less affected at P0, P0.4, and P0.8. Clearly, [P] became higher with increasing P supply. Interestingly, [P] in the leaves (including young leaves and remaining leaves) and stems increased linearly with P supply (Figure 2.1.10A and B), whereas it increased nonlinearly in roots and tubers (Figure 2.1.10C and D). This could be explained by P distribution in the plant. Because P plays important roles in photosynthesis (Carstensen et al., 2018; Zhang et al., 2014), it is generally transported to the storage pools in vacuoles and other organelles, such as chloroplasts, where the photosynthetic machinery is located.

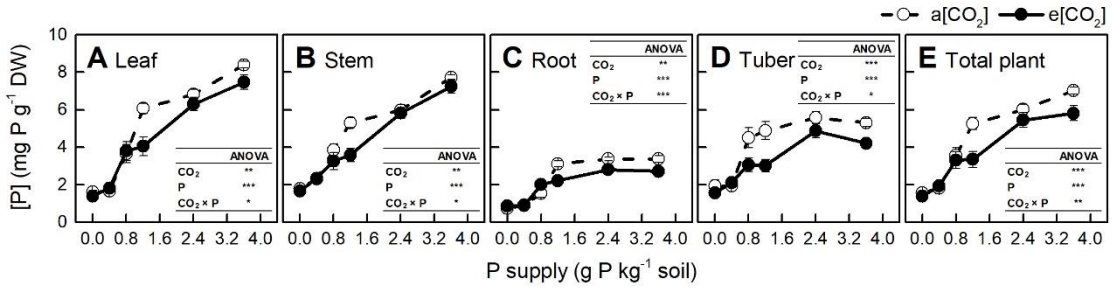

Figure 2.1.10 P concentration ([P]) of several organs of potato plants grown under a[CO_2] (439 ± 9 ppm) and e[CO_2] (825 ± 17 ppm) with different P supply rates (0, 0.4, 0.8, 1.2, 2.4, and 3.6 g P kg^{-1} soil). Data in each plot are means ± S.E. (n = 6 or 5 biological replicates for each treatment). Statistical comparisons (two-way ANOVA) between CO_2 concentrations and P supply rates as well as their interaction (CO_2 × P) are presented. **(A)** Leaf [P]; **(B)** stem [P]; **(C)** root [P]; **(D)** tuber [P]; and **(E)** total plant [P].

Total plant P content was higher under e[CO_2] than under a[CO_2] (Figure 2.1.11A). PAE and PUE in potato plants were enhanced by e[CO_2] (Figure 2.1.11B and C).

At harvest, root parameters were measured to evaluate root function for P uptake in potato plants. The results showed that root length was decreased, whereas root surface area was not changed by e[CO_2] (Figure 2.1.12). Neither of these parameters could explain the increase in PAE under e[CO_2].

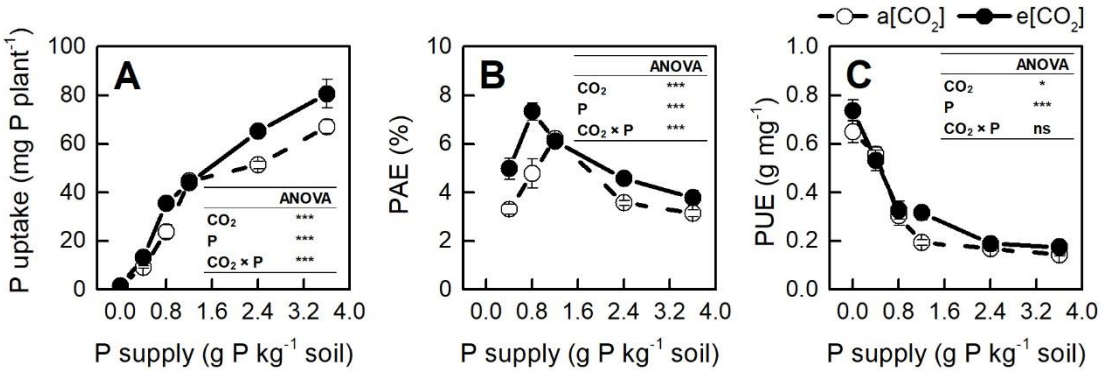

Figure 2.1.11 (A) P uptake, **(B) P** acquisition efficiency (PAE), and **(C)** P utilization efficiency (PUE) of potato plants grown under a[CO_2] (439 ± 9 ppm) and e[CO_2] (825 ± 17 ppm) with different P supply rates (0, 0.4, 0.8, 1.2, 2.4, and 3.6 g P kg^{-1} soil). Data in each plot are means ± S.E. (n = 6 or 5 biological replicates for each treatment). Statistical comparisons (two-way ANOVA) between CO_2 concentrations and P supply rates as well as their interaction (CO_2 × P) are presented. PAE (%) = (P content in P-treated plant – P content in P0 plant) × 100% (g)/ P supply (g). PUE (g mg^{-1}) = total plant biomass (g)/ total plant P content (mg).

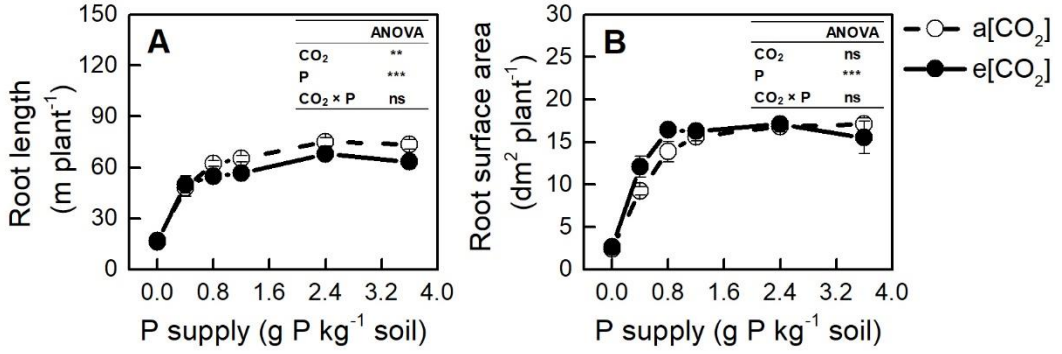

Figure 2.1.12 (A) Root length and **(B)** root surface area of potato plants grown under a[CO_2] (439 ± 9 ppm) and e[CO_2] (825 ± 17 ppm) with different P supply rates (0, 0.4, 0.8, 1.2, 2.4, and 3.6 g P kg^{-1} soil). Data in each plot are means ± S.E. (n = 6 or 5 biological replicates for each treatment). Statistical comparisons (two-way ANOVA) between CO_2 concentrations and P supply rates as well as their interaction (CO_2 × P) are presented.

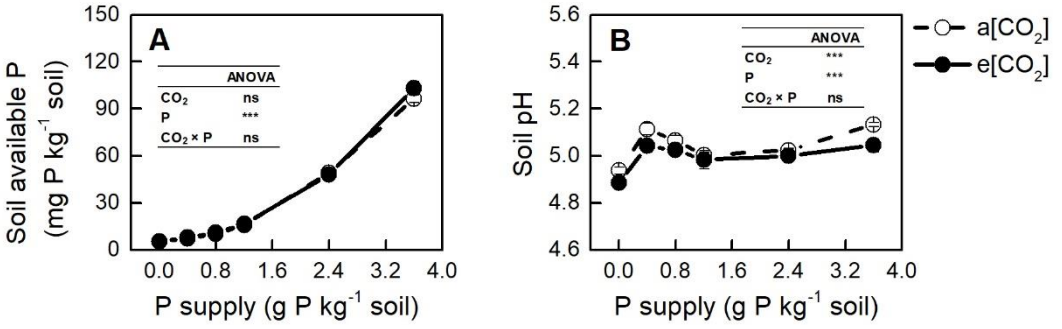

Figure 2.1.13 (A) Soil available P and **(B)** soil pH after harvesting under a[CO_2] (439 ± 9 ppm) and e[CO_2] (825 ± 17 ppm) with different P supply rates (0, 0.4, 0.8, 1.2, 2.4, and 3.6 g P kg^{-1} soil). Data in each plot are means ± S.E. (n = 6 biological replicates for each treatment). Statistical comparisons (two-way ANOVA) between CO_2 concentrations and P supply rates as well as their interaction (CO_2 × P) are presented.

Due to the increased amount of P under e[CO_2], soil available P (T_{RUOG}-P) may be lower under e[CO_2]. However, this result suggested that there was no significant difference in soil T_{RUOG}-P between the two CO_2 conditions (Figure 2.1.13A). In addition, soil pH under e[CO_2] was lower than that under a[CO_2] (Figure 2.1.13B). Thus, soil pH might contribute to sustain soil P availability, thereby enhancing PAE under e[CO_2].

2.1.3 Discussion

Tissue [P] in potato plants generally ranges between 1 to 4 mg P g^{-1} dry mass (Jenkins and Mahmood, 2003; White et al., 2018). In the present study, [P] varied among plant organs, varying from 0.75 to 8.40 mg P g^{-1} dry mass, depending on growth conditions (Figure. 2.1.10). This wide range indicated that P supply levels fully covered P-deficient and P-sufficient conditions. P deficiency was reported to increase starch in leaves by affecting photosynthetic electron transport chain (Carstensen et al., 2018). Consistently, I detected a negative correlation of starch concentration with [P] in leaves across each CO_2 (Figure 2.1.5B), suggesting that starch accumulation responded more strongly to foliar [P] than to [CO_2]. A similar consistency with previous researches (Ainsworth et al., 2002; Jauregui et al., 2018; Katny et al., 2005) was found for leaf starch, which increased under e[CO_2] (Figure 2.1.5A), and may be explained by the decrease in [P] observed under e[CO_2] (Figure 2.1.10).

1.5-fold increase in maximum total biomass by e[CO_2] followed a phosphorus supply dependent pattern

In contrast to Fleisher et al. (2012, 2013b), who did not find a significant interaction between CO_2 and P supply in potato, our study clearly demonstrated significant CO_2 – P-supply interaction effects on organ biomass (Figure 2.1.3) and WUE (Figure 2.1.7B), but not on water use (Figure 2.1.7A). The difference between these contradictory results may be related to the growing conditions and potato cultivar used in these studies. The strong interaction in this study clearly indicated that e[CO_2] effects were greatly dependent on P supply. Thus, maximum biomass enhancement by CO_2 enrichment was evident only when P demand was fully supplied, an effect that involved enhanced WUE, but not water use. Moreover, both biomass and WUE clearly described a saturation-kinetics pattern when plotted against P supply, hence enabling an accurate estimation of minimum P supply rate to reach maximum performance. To my knowledge, this is the first report of a saturation response-curve against P supply rate under different CO_2

conditions in potato plants. Therefore, maximum plant total biomass under e[CO_2] was achieved even without additional P or water supply, despite the 1.5-fold biomass increase relative to biomass accumulation under a[CO_2].

Because e[CO_2] can also affect plant respiration (Gonzalez-Meler et al., 2004), carbon use efficiency, which is defined as the ratio of net primary production to gross primary production, is important for understanding CO_2-fertilization effects (Long et al., 2006). Gong et al. (2017) reported the reduced inhibition of leaf respiration by light and the diminished leaf mass ratio as the two main process contributing to the reduction of carbon use efficiency under e[CO_2]. Consistent with previous studies (Norby et al., 2004; Gong et al., 2017), the potato plants examined in this investigation developed a lower leaf (source) mass ratio and a higher tuber (sink) mass ratio (Figure 2.1.4). Although leaf respiration was not measured in our study, leaf starch concentration was higher under e[CO_2] than under a[CO_2] (Figure 2.1.5A). Amthor (2000) reported that accumulation of nonstructural carbohydrates in source leaves may stimulate respiratory activity through increased phloem loading and translocation. Therefore, carbon use efficiency in potato plants could be decreased at e[CO_2] through the decrease in leaf mass ratio and increase in leaf respiration, as detected in the present study.

Phosphorus supply requirement for maximum total biomass was unchanged by e[CO_2]

Generally, plants growing under e[CO_2] are expected to require a larger P supply to take full advantage of the CO_2 enrichment (Pang et al., 2018). Consistently, Rogers et al. (1993) found that, on a foliar mass basis, critical [P] increased under e[CO_2] in cotton and wheat. By contrast, our data showed a similar critical [P] (approximately 110 mg P m^{-2}) under the two CO_2 conditions tested (Figure 2.1.9). Although total P uptake was higher under e[CO_2] compared to that under a[CO_2] (Figure 2.1.11A), P supply requirement for maximum growth was unchanged by e[CO_2] (Figure 2.1.3) because of higher PAE (Figure 2.1.11B) or PUE (Figure 2.1.11C). Thus, the maximum biomass

under both [CO_2] was obtained at the same P supply rate (1.2 g P kg^{-1} soil) (Figure 2.1.3F). Notably, plant P content at P1.2 (which allowed maximum plant growth) was similar regardless of [CO_2] (Figure 2.1.11A). Nevertheless e[CO_2] allowed 1.5-fold higher plant total biomass than a[CO_2], thus resulting in higher PUE (Figure 2.1.11C).

Root growth and morphology greatly affect the amount of P extracted from the soil because of P bioavailability constraints. Silberbush and Barber (1983) demonstrated that in soils with low P availability, root length was an excellent predictor of P content in some plant species. However, in the present study, e[CO_2] increased PAE in potato plants not by increasing root length or root surface area (Figure 2.1.12). Therefore, I hypothesized that the increase in PAE observed in the potato plants used here was likely due to a lowering of soil pH under e[CO_2] through a change in the rhizosphere. In fact, rhizosphere acidification might increase the concentration of phosphate in the rhizosphere by increasing the desorption of phosphate from the soil solid phase (Hedley et al., 1982). Whether rhizosphere acidification might result from enhanced root or microbial activity remains unknown.

e[CO_2]-induced increase in water-use efficiency was phosphorus dependent

The results indicate that under e[CO_2], WUE increased relative to a[CO_2] in a P-dependent manner (Figure 2.1.7B). As drought stress is a serious limitation for plant growth in many cropping regions of the world, it is interesting that e[CO_2] may be more than palliative to allow increased WUE without growth reduction, especially in C_3 plants (Brouder and Volenec, 2008). In the present study, not only e[CO_2], but also P supply may improve WUE by a strong interaction with CO_2 enrichment (P = 0.010).

Increased WUE induced by e[CO_2] was due to an increase in dry weight rather than a decrease in water loss (Figures 2.1.3F and 2.1.7B). Leaf area was slightly larger in e[CO_2] than in a[CO_2] at 0.4, 0.8, and 2.4 P supply levels and similar among the remaining treatments (Figure 2.1.8A) while P levels had a major effect on this parameter. These results likely indicate that the effect of CO_2 enrichment on leaf area

was weaker than that of P supply level, although both main effects as well as their interaction were significant. However, a larger leaf area under e[CO_2] without a concomitant increase in water loss may imply a decrease in g_s in potato plants (Figure 2.1.8C). By contrast, P fertilization may help optimize WUE by adjusting both g_s and stomatal density. I measured productive WUE (i.e. dry matter production per unit water consumption), which is more informative for agricultural and ecological purposes, than instantaneous WUE (Sinclair et al., 1984). Here, g_s decreased under e[CO_2] and also with increasing P supply rate (Figure 2.1.8C). Similarly, stomatal density was also responsive to P supply rate, although it remained unaffected by CO_2 enrichment (Figure 2.1.8B). It is likely that potato plants may save water by decreasing g_s while simultaneously enhancing gas exchange by increasing stomatal density at P supply rates, thereby ultimately improving WUE. However, the relationship between stomatal density, g_s and water use under different P supply regimes may be complex and should be examined in future investigations.

2.1.4 Conclusions

The present study aimed at clarifying the following aspects: 1) to what extent can maximum biomass accumulation be enhanced by e[CO_2] in potato plants?, 2) how much P is required to achieve maximum biomass accumulation and will e[CO_2] increase the plant P requirement?, and finally 3) how does e[CO_2] affect water consumption by the plant to reach maximum biomass accumulation under varying P supply? After carrying out trials, I found 1) a 1.5-fold maximum biomass increase under e[CO_2], relative to biomass accumulation under a[CO_2]. However, 2) P requirement for maximum potato plant growth was not affected by CO_2 enrichment as shown by a similar critical [P], and 3) total water consumption was not affected by [CO_2] regardless of great differences in plant total biomass because of higher WUE.

The interaction between CO_2 and P supply provides a sound theoretical basis for P fertilizer management under e[CO_2] conditions. As the response to CO_2 enrichment may vary with different growing conditions and plant species (Jin et al., 2015), further research is needed to elucidate the mechanism underlying the interaction between CO_2 and P supply. The P crisis around the world is becoming an increasingly serious problem (Vaccari, 2009); therefore, to avoid wasteful and environmentally harmful P application in agricultural soils, and to maximize plant P acquisition efficiency, a more sustainable paradigm in P fertilizer management should be devised, accounting for climate change, including localized-P supply (Kume et al., 2006), and arbuscular mycorrhizal fungi symbiosis (Smith et al., 2011).

Chapter 2.2

Trial N

Nitrogen and water demands for maximum growth of *Solanum tuberosum* under doubled CO_2: interaction with phosphorus based on the demands

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2.2.1 Materials and Methods

Experimental design and growing conditions

A pot experiment was carried out in GC, with the same setting in Chapter 2.1. The actual conditions in the chambers were monitored every 5 min (Figure 2.2.1).

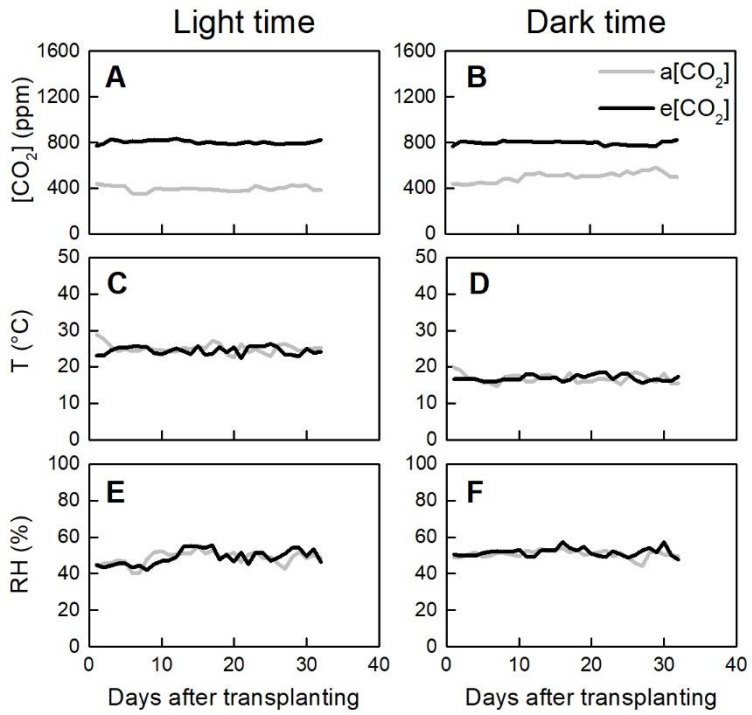

Figure 2.2.1 Actual conditions in growth chambers during growth period (33 days after transplanting). Actual CO_2 concentrations at light time **(A)** and dark time **(B)**. Actual temperature (T) at light time **(C)** and dark time **(D)**. Actual relative humidity (RH) at light time **(E)** and dark time **(F)**.

Naturally sprouted potato tubers ('Irish Cobbler') were transplanted into 1-L pots (diameter, 11.3 cm; depth, 14 cm; one plant per pot) filled with 580 g of dry andosol. Before transplanting, K (1.6 g K_2O kg^{-1} dry soil) was uniformly mixed with the soil in the form of potassium chloride (60.0% K_2O). Calcium superphosphate (17.5% P_2O_5) was uniformly mixed with the soil to control P rates at 0.3 (low P, LP) and 3 (high P, HP) g P kg^{-1} dry soil. Urea (46.0% N) was uniformly mixed with the soil to control N supply rates at 0, 0.2, 0.4, 0.8, or 1.6 g N kg^{-1} of dry soil (hereafter, these treatments are designated as N0, N0.2, N0.4, N0.8, and N1.6, respectively). Soil water condition was kept at approximately 80% (w/w) by weighing pots and supplementing water. The

experiment was organized following a factorial design (two CO_2 concentrations × two P supply rates × five N supply rates) with four biological replicates.

Stomatal conductance and stomatal density

One day before harvest, the g_s of the youngest fully expanded leaf was measured on the adaxial surface between 8:00 and 12:00 in the morning with a leaf porometer (SC-1, Decagon Devices Inc., USA). Immediately after the measurement of stomatal conductance, the same leaves were coated with nail polish; next, imprints were taken from each leaf and mounted on a glass microscope slide for stomatal density count in the same way in Chapter 2.1.

Harvest and sampling

All plants were harvested on the 33 DAT. Before harvest, the youngest fully expanded leaf was sampled, between 8:00 and 10:00 in the morning, from each plant for starch quantification. Sampled leaves were immediately frozen in liquid N. After leaf area was analyzed, the samples were dried for starch analysis. At harvest, the remaining leaves, stems, roots, and tubers were separated and dried in an oven at 80 °C to a constant mass for dry weight determination. All samples were then ground to powder for N quantification.

Critical nitrogen concentration

Simple regressions were analyzed in Origin 9.0 (https://www.originlab.com) to calculate critical [N] on mass and area basis. Critical [N] is defined as the minimum [N] in the crop required to reach 90% of maximum growth (Chisholm et al., 1981; Conroy, 1992). Coefficients of the polynomial equations ($y = a + bx + cx^2$) from regression relations between foliar [N] (x) and total biomass (y) were used to calculate critical [N] as follows,

$$\text{Critical [N]} = \frac{-b + \sqrt{b^2 - (4ac + 9b^2)/10}}{2c}$$

N uptake, N acquisition efficiency and N utilization efficiency

N uptake was defined as the total N content in the plant. According to Vos (1997), N acquisition efficiency (NAE) was calculated as follows,

NAE (%) = (N content in N-treated plant – N content in N0 plant) × 100% (g)/ N supply (g).

N utilization efficiency (NUE) was calculated following Hirose (2011),

NUE (g mg^{-1}) = Total plant biomass (g)/ Total plant N content (mg).

Statistical analysis

The experiment was organized following a factorial design with two CO_2 concentrations, two P supply, and five N supply rates with four biological replicates, data are expressed as mean ± standard error (S.E.) for the four biological replicates. Data were analyzed in SPSS 16.0 (SPSS Inc., Chicago, IL., USA) using three-way ANOVA at the 0.05 probability level (Table 2.2.1). Considering the significant interactions between CO_2 concentration and P supply rate from some of the main measured items, a two-way ANOVA was used to analyze the interaction between CO_2 concentration and N supply rate at each P supply rate.

Table 2.2.1 Output of three-way ANOVA for the measurements of potato plants grown under a[CO_2] (395 ± 4 ppm) and e[CO_2] (802 ± 3 ppm) with different N supply rates (0, 0.2, 0.4, 0.8, and 1.6 g N kg^{-1} soil) at Low P (LP, 0.3 g P kg^{-1} soil) and High P (HP, 3 g P kg^{-1} soil).

	CO_2	P	N	CO_2 × P	CO_2 × N	P × N	CO_2 × P × N
Leaf biomass	< 0.001	< 0.001	< 0.001	0.041	0.172	< 0.001	0.082
Stem biomass	< 0.001	< 0.001	< 0.001	0.005	0.004	< 0.001	0.003
Root biomass	< 0.001	0.105	< 0.001	< 0.001	0.064	0.142	0.001
Tuber biomass	0.002	0.010	< 0.001	0.369	0.279	0.002	0.257
Total plant biomass	< 0.001	< 0.001	< 0.001	< 0.001	0.001	< 0.001	0.187
Starch concentration	< 0.001	< 0.001	< 0.001	0.405	< 0.001	0.456	0.044
Leaf mass per area	< 0.001	< 0.001	< 0.001	0.116	0.010	0.761	0.023
Water use	0.016	< 0.001	< 0.001	0.005	< 0.001	< 0.001	0.547
Water-use efficiency	< 0.001	0.010	< 0.001	0.239	0.284	0.055	0.494
Leaf area	0.050	< 0.001	< 0.001	0.008	0.005	< 0.001	0.625
Stomatal conductance	< 0.001	0.020	< 0.001	0.224	0.089	0.019	0.878
Stomatal density	0.057	0.713	0.001	0.771	0.376	0.201	0.499
Foliar $[N]_{mass}$	< 0.001	< 0.001	< 0.001	0.289	< 0.001	< 0.001	0.503
Foliar $[N]_{area}$	0.082	0.291	< 0.001	0.158	0.002	0.077	0.012
N uptake	< 0.001	< 0.001	< 0.001	0.015	< 0.001	< 0.001	< 0.001
N acquisition efficiency	0.670	< 0.001	< 0.001	0.919	< 0.001	< 0.001	< 0.001
N utilization efficiency	< 0.001	< 0.001	< 0.001	0.035	0.009	0.267	0.088
Foliar $[P]_{area}$	< 0.001	< 0.001	< 0.001	0.533	< 0.001	< 0.001	0.019

2.2.2 Results

Plant growth and biomass

Based on the appearance, especially leaf color, plant growth was significantly affected by N supply, and it also appeared to be somewhat affected by both CO_2 level and P supply (Figure 2.2.2). It should be noted that at earlier growth stages, salinity stress was observed under high N supply rates, especially under LP conditions (Figure 2.2.3).

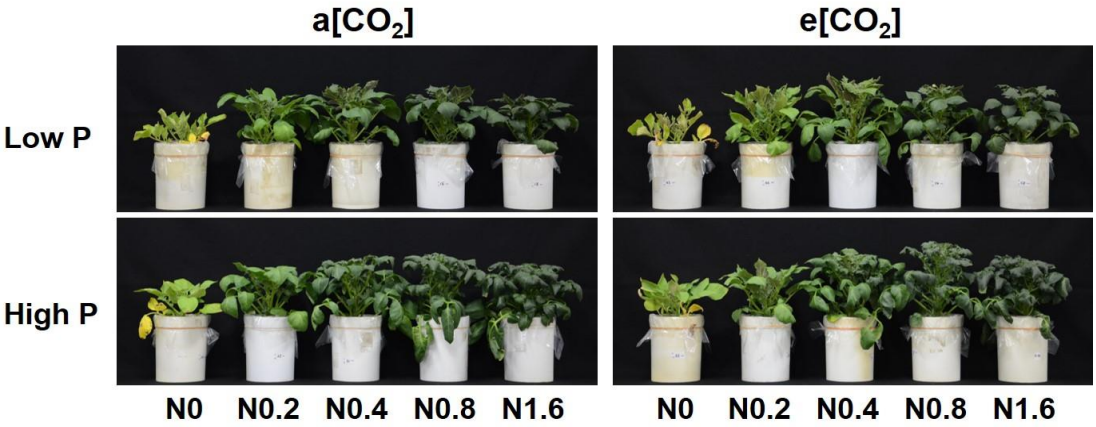

Figure 2.2.2 Appearance of potato plants at harvest (33 days after transplanting).

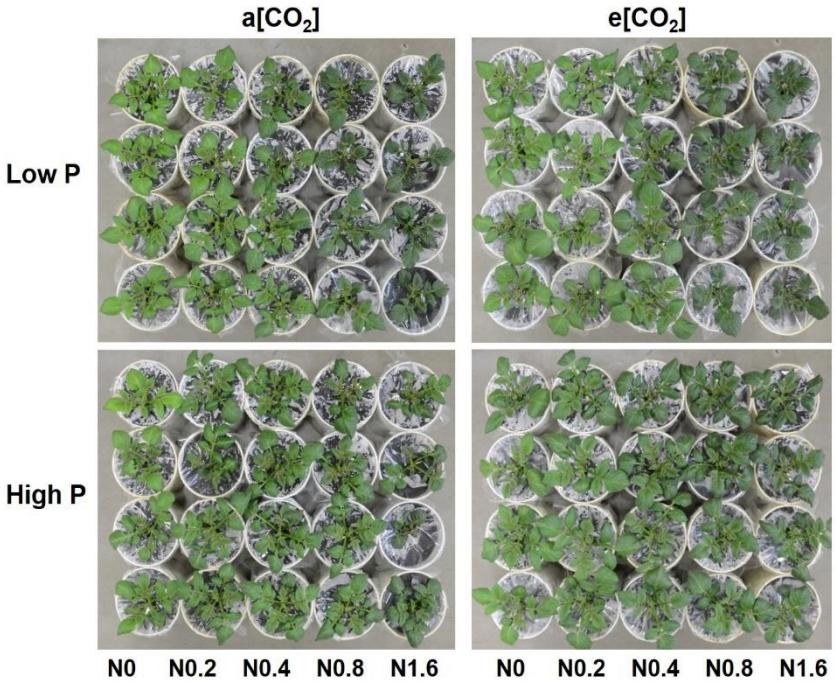

Figure 2.2.3 Appearance of potato plants at 8 days after transplanting.

With e[CO_2], increased biomass of leaves, stems, roots, and tubers occurred at each N supply rate under HP, whereas e[CO_2] increased biomass only in leaves ($P < 0.001$) and stems ($P = 0.009$) under LP where clear increases were observed only at some certain N supply rates (N0.4 and N0.8) (Figure 2.2.4). Despite the higher tuber biomass under e[CO_2] comparing with a[CO_2] at high N supply (N0.8 and N1.6) under LP, effects of e[CO_2] on tuber biomass was not significant ($P = 0.115$). Biomass of leaves, stems, roots, and total plant increased with increases in N supply to a certain range and then decreased, especially under LP. Tuber biomass decreased with the increases in N supply. Furthermore, the maximum plant growth under different P supply rates were obtained at different N supply rates (Figure 2.2.4E and J). Maximum biomass was achieved at N0.4 in LP under both CO_2 conditions, whereas it was achieved at N0.8 and N1.6 under a[CO_2] and e[CO_2], respectively, in HP.

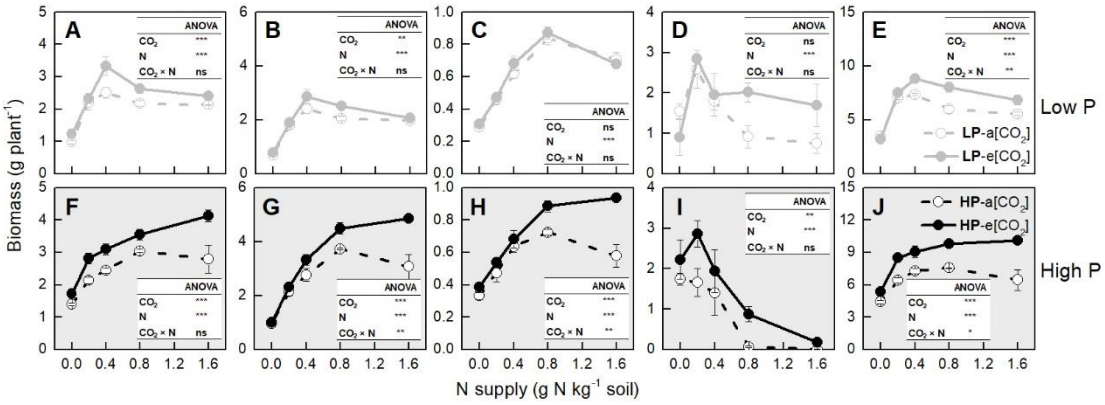

Figure 2.2.4 Biomass of several organs of potato plants grown under a[CO_2] (395 ± 4 ppm) and e[CO_2] (802 ± 3 ppm) at Low P (LP, 0.3 g P kg^{-1} soil) and High P (HP, 3 g P kg^{-1} soil) with different N supply rates (0, 0.2, 0.4, 0.8, and 1.6 g N kg^{-1} soil). Data in each plot are means ± S.E. (n = 4 biological replicates for each treatment). Statistical comparisons (two-way ANOVA) between CO_2 concentrations and N supply rates as well as their interaction (CO_2 × N) are presented. **(A) (F)** Leaf biomass; **(B) (G)** stem biomass; **(C) (H)** root biomass; **(D) (I)** tuber biomass; and **(E) (J)** total plant biomass.

Biomass partitioning

Under LP, e[CO_2] did not alter tuber proportion, however, under HP there were increased biomass allocation to the tuber (Figure 2.2.5). To the contrary, high N supply decreased biomass allocation to tuber under both CO_2 and P supply (Figure 2.2.5). Compared with LP, HP decreased tuber proportion under both CO_2 conditions, especially at a[CO_2] (Figure 2.2.5).

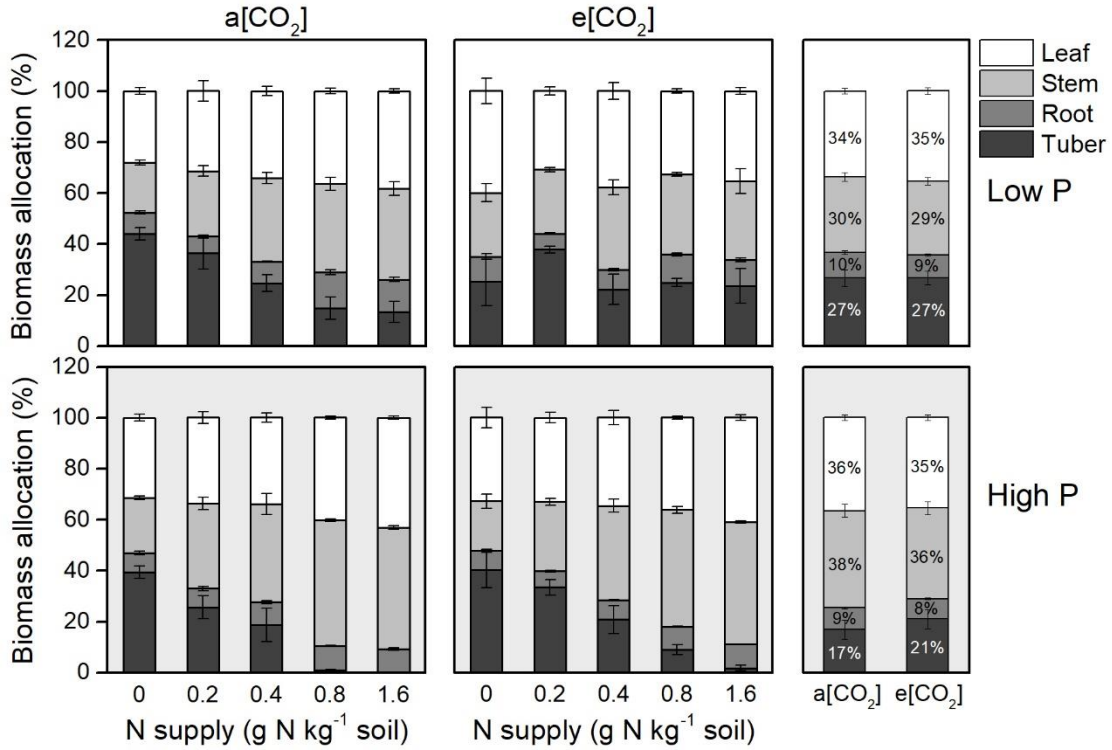

Figure 2.2.5 Biomass partitioning in organs of potato plants grown under a[CO_2] (395 ± 4 ppm) and e[CO_2] (802 ± 3 ppm) at Low P (LP, 0.3 g P kg^{-1} soil) and High P (HP, 3 g P kg^{-1} soil) with different N supply rates (0, 0.2, 0.4, 0.8, and 1.6 g N kg^{-1} soil). Data in each plot are means ± S.E. (n = 4 biological replicates for each treatment).

Starch and leaf mass per area

There was a general tendency that starch concentration in the youngest expanded leaf had the highest peak and decreased along with increases of N supply excepting N1.6, but the changing patterns were different by CO_2 conditions as the significant interaction effects between them were detected under both P supply rates (Figure 2.2.6A and C). The highest peak of the starch concentration appeared at higher N supply level under

e[CO_2] than a[CO_2] at both P supply, and the highest peak was observed at lower N supply level under HP than LP within each [CO_2].

Consistent with starch concentration, leaf mass per area (LMA) of the youngest expanded leaf was higher under e[CO_2] and low N supply compared to those under a[CO_2] and high N supply (Figure 2.2.6B and D). A clear correlation between starch and LMA was observed (Figure 2.2.6E).

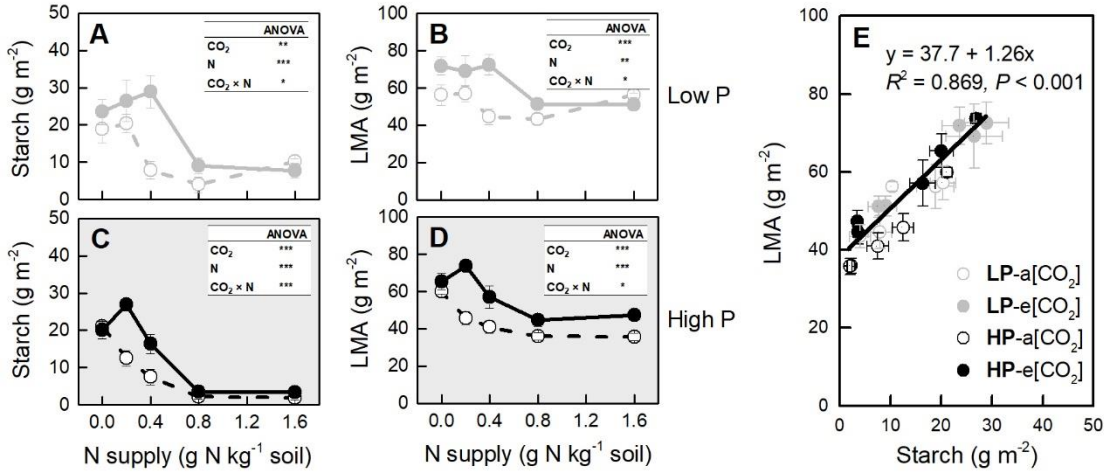

Figure 2.2.6 (A) (C) Starch concentration and **(B) (D)** leaf mass per area (LMA) of the youngest fully expanded leaf in potato plants grown under a[CO_2] (395 ± 4 ppm) and e[CO_2] (802 ± 3 ppm) at Low P (LP, 0.3 g P kg^{-1} soil) and High P (HP, 3 g P kg^{-1} soil) with different N supply rates (0, 0.2, 0.4, 0.8, and 1.6 g N kg^{-1} soil). Data in each plot are means ± S.E. (n = 4 biological replicates for each treatment). Statistical comparisons (two-way ANOVA) between CO_2 concentrations and N supply rates as well as their interaction (CO_2 × N) are presented. **(E)** Relation between starch concentration and LMA of the youngest fully expanded leaf. Regression is as follows: $y = 37.7 + 1.26x$, R^2 = 0.869, P < 0.001.

Water use and water-use efficiency

I monitored time-course changes in cumulative transpiration as water use in potato plants (Figure 2.2.7).

The interaction effect between CO_2 and N supply on water use was significant under both LP (P = 0.045) and HP (P = 0.028), indicating the change of water use with N supply was different by CO_2 conditions (Figure 2.2.8A and C). Specifically, water use was lower in e[CO_2] than a[CO_2] at lower N supply rates, whereas at higher N

supply rates, water use was similar (N1.6 at LP and N0.8 at HP) otherwise became higher (N 1.6 at HP) in e[CO_2]. High N supply increased water use but from N0.4 under LP, water use gradually decreased.

A significant increase in WUE by e[CO_2] at each N supply rate was observed under both P supply rates (Figure 2.2.8B and D). However, WUE decreased with increases in N supply until N0.8 and then keep unchanging under both CO_2 conditions with HP supply rate, or slightly increasing under a[CO_2] with LP supply rate.

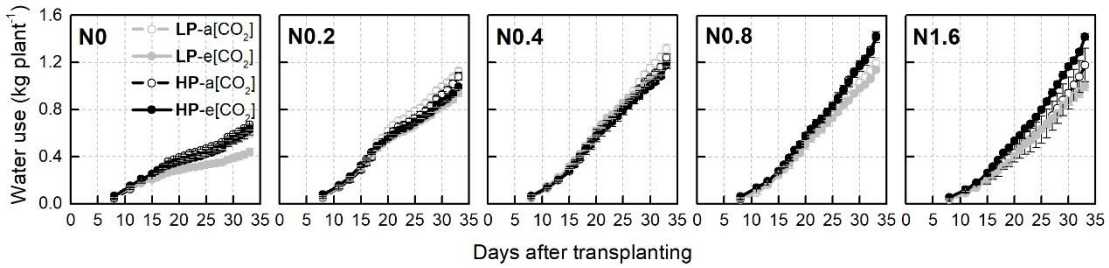

Figure 2.2.7 Water use by potato plants over the experimental period (33 days after transplanting) under a[CO_2] (395 ± 4 ppm) and e[CO_2] (802 ± 3 ppm) at Low P (LP, 0.3 g P kg^{-1} soil) and High P (HP, 3 g P kg^{-1} soil) with different N supply rates (0, 0.2, 0.4, 0.8, and 1.6 g N kg^{-1} soil). Data in each plot are means ± S.E. (n = 4 biological replicates for each treatment).

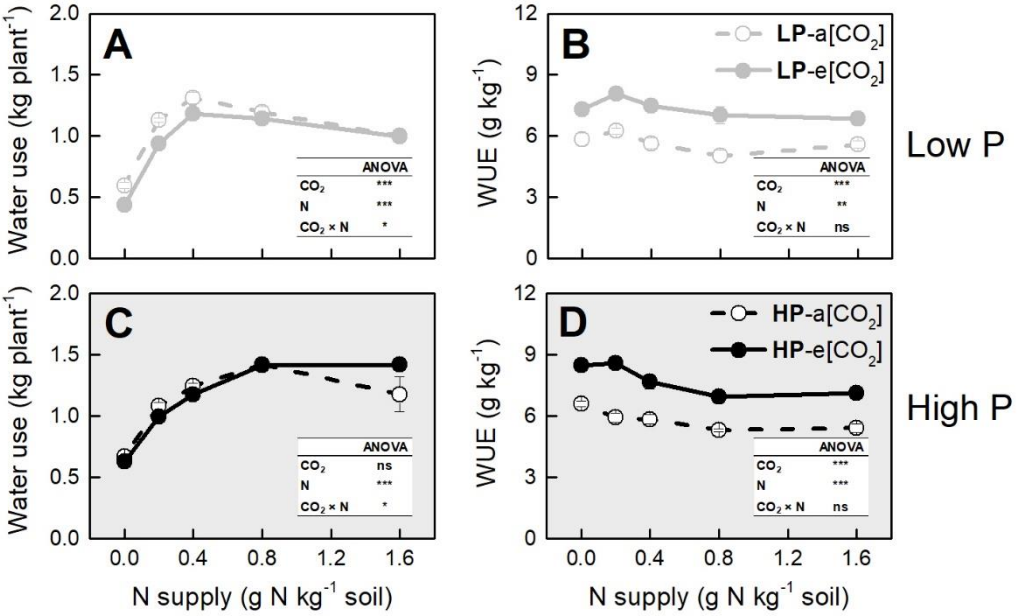

Figure 2.2.8 (A) (C) Water use and **(B) (D)** water-use efficiency (WUE) of potato plants grown under a[CO_2] (395 ± 4 ppm) and e[CO_2] (802 ± 3 ppm) at Low P (LP, 0.3 g P kg^{-1} soil) and High P (HP, 3 g P kg^{-1} soil) with different N supply rates (0, 0.2, 0.4, 0.8, and 1.6 g N kg^{-1} soil). Data in each plot are means ± S.E. (n = 4 biological replicates for each treatment). Statistical comparisons (two-way ANOVA) between CO_2 concentrations and N supply rates as well as their interaction (CO_2 × N) are presented.

Leaf area, stomatal conductance, and stomatal density

The most important factors affecting water use in potato plants, leaf area, g_s, as well as stomatal density were examined. Leaf area was not affected by e[CO_2] under LP (Figure 2.2.9A). Two-way ANOVA results suggest that e[CO_2] decreased g_s but did not change stomatal density (Figure 2.2.9B and C), which indicates the decreased water use by e[CO_2] under LP was attributed to decreased g_s. Under HP, e[CO_2] slightly increased leaf area (Figure 2.2.9D) without increase in water use (Figure 2.2.8C), which was also related to g_s as a clear decrease in g_s under e[CO_2] was observed while stomatal density was not changed (Figure 2.2.9E and F).

Changes in leaf area and water use with an increase in N supply were similar (Figures 2.2.8 and 2.2.9). I found a clear positive correlation between leaf area and water use (Figure 2.2.10). Additionally, g_s was little affected by N supply under LP (P = 0.064), but increased with the increases in N supply under HP (P = 0.002) (Figure 2.2.9). High N supply increased stomatal density under HP, but the effects of N supply became complicated under LP (Figure 2.2.9).

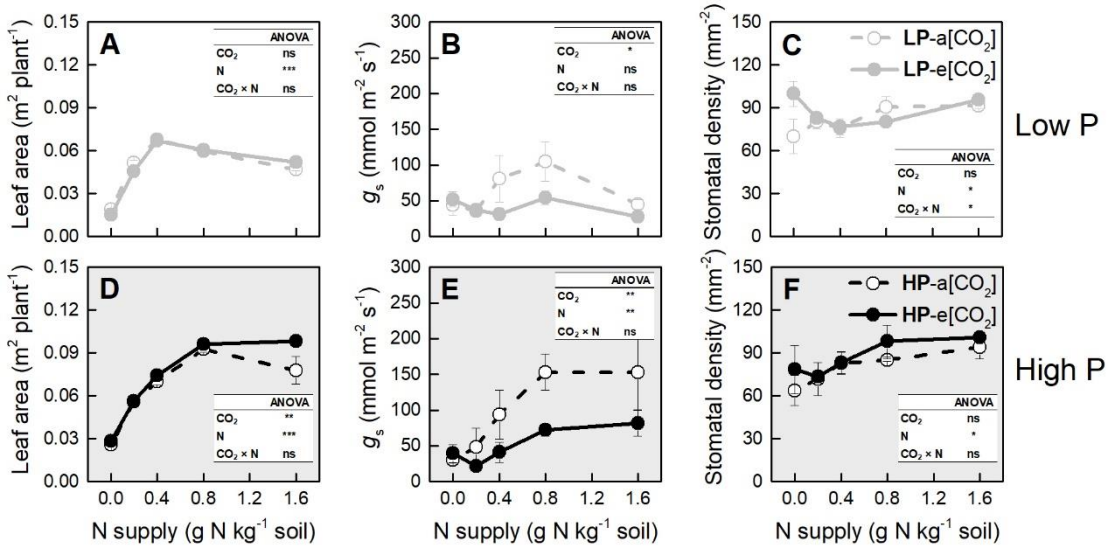

Figure 2.2.9 (A) (D) Leaf area, **(B) (E)** stomatal conductance (g_s), and **(C) (F)** stomatal density of potato plants grown under a[CO_2] (395 ± 4 ppm) and e[CO_2] (802 ± 3 ppm) at Low P (LP, 0.3 g P kg^{-1} soil) and High P (HP, 3 g P kg^{-1} soil) with different N supply rates (0, 0.2, 0.4, 0.8, and 1.6 g N kg^{-1} soil). Data in each plot are means ± S.E. (n = 4 biological replicates for each treatment). Statistical comparisons (two-way ANOVA) between CO_2 concentrations and N supply rates as well as their interaction (CO_2 × N) are presented.

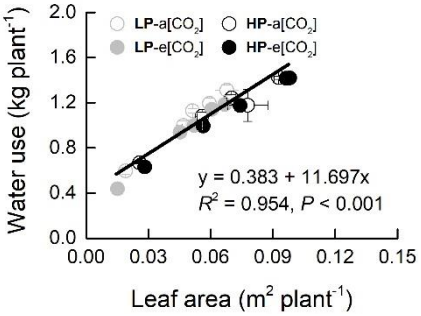

Figure 2.2.10 Relation between leaf area and water use in potato plants grown under a[CO_2] (395 ± 4 ppm) and e[CO_2] (802 ± 3 ppm) at Low P (LP, 0.3 g P kg^{-1} soil) and High P (HP, 3 g P kg^{-1} soil) with different N supply rates (0, 0.2, 0.4, 0.8, and 1.6 g N kg^{-1} soil). Data in each plot are means ± S.E. (n = 4 biological replicates for each treatment). Regression is as follows: $y = 0.383 + 11.679x$, $R^2 = 0.954$, $P < 0.001$.

Foliar N concentration

As LMA markedly changed (Figure 2.2.6B and D), foliar N concentration was calculated based on mass and area. When N concentration was calculated based on mass, CO_2 effects were clearly observed under both P supply rates ($P < 0.001$) (Figure 2.2.11A and C). However, the effects of CO_2 were reduced under LP ($P = 0.041$) and disappeared under HP ($P = 0.799$) when N concentration was calculated based on area (Figure 2.2.11B and D).

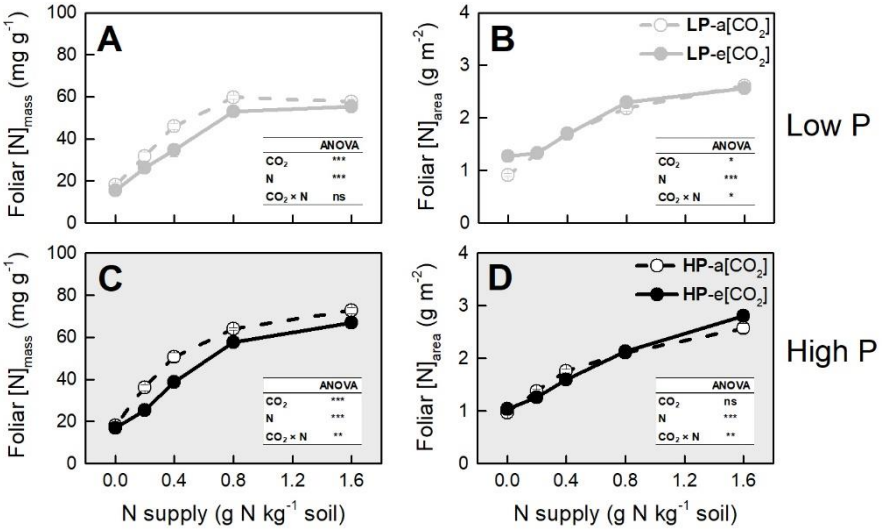

Figure 2.2.11 Foliar N concentration on mass basis ($[N]_{mass}$) **(A) (C)**, and area basis ($[N]_{area}$) **(B) (D)** of potato plants grown under a[CO_2] (395 ± 4 ppm) and e[CO_2] (802 ± 3 ppm) at Low P (LP, 0.3 g P kg^{-1} soil) and High P (HP, 3 g P kg^{-1} soil) with different N supply rates (0, 0.2, 0.4, 0.8, and 1.6 g N kg^{-1} soil). Data in each plot are means ± S.E. (n = 4 biological replicates for each treatment). Statistical comparisons (two-way ANOVA) between CO_2 concentrations and N supply rates as well as their interaction (CO_2 × N) are presented.

N uptake, N acquisition efficiency and N utilization efficiency

N uptake was increased by e[CO_2] when N supply was high particularly at N0.8 under LP and N 1.6 under HP, as supported by the strong interaction effect ($P < 0.001$) between CO_2 and N supply under both P supply rates (Figure 2.2.12A and D). The effect of CO_2 on NAE was also dependent on N supply rate due to the significant interaction effect between them under both P supply rates; e[CO_2] decreased the NAE with low N supply, whereas that increased the efficiency with high N supply (Figure 2.2.12B and E). The trend was more remarkable under HP comparing to LP. NUE was increased by e[CO_2] under HP (Figure 2.2.12F), but it was not affected by e[CO_2] under LP (Figure 2.2.12C). As for the effects of N supply, N uptake increased with an increase in N supply, especially under HP. On the contrary, NAE and NUE decreased with an increase in N supply under both P supply rates.

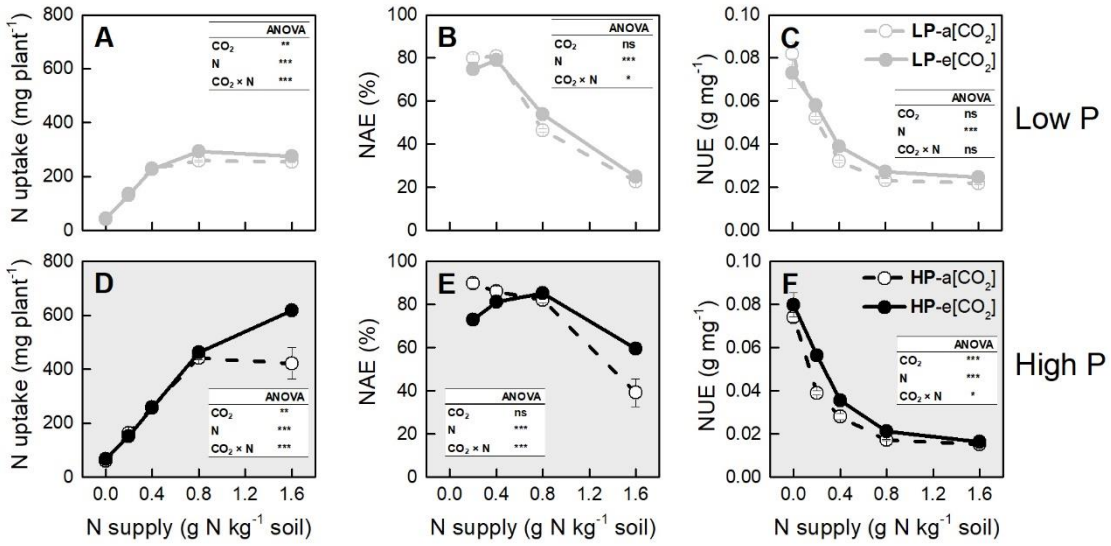

Figure 2.2.12 (A) (B) N uptake, **(C) (D)** N acquisition efficiency (NAE), and **(E) (F)** N utilization efficiency (NUE) of potato plants grown under a[CO_2] (395 ± 4 ppm) and e[CO_2] (802 ± 3 ppm) at Low P (LP, 0.3 g P kg^{-1} soil) and High P (HP, 3 g P kg^{-1} soil) with different N supply rates (0, 0.2, 0.4, 0.8, and 1.6 g N kg^{-1} soil). Data in each plot are means ± S.E. (n = 4 biological replicates for each treatment). Statistical comparisons (two-way ANOVA) between CO_2 concentrations and N supply rates as well as their interaction (CO_2 × N) are presented. NAE (%) = (N content in N-treated plant – N content in N0 plant) × 100% (g)/ N supply (g). NUE (g mg^{-1}) = Total plant biomass (g)/ Total plant N content (mg).

Critical N concentration

To evaluate N demand in potato plant growth, I analyzed critical [N], the minimum [N] required to achieve 90% maximum growth. I calculated critical [N] in two ways, that is foliar [N] per dry weight ($[N]_{mass}$) and foliar [N] per area ($[N]_{area}$). The two different calculation methods produced inconsistent results. Under LP condition, critical $[N]_{mass}$ and $[N]_{area}$ under both CO_2 showed similar values, 30 mg N g^{-1} and 1.43 g N m^{-2}, respectively (Figure 2.2.13A and B). Under HP, however, critical $[N]_{mass}$ decreased under e[CO_2] from 41.3 to 34.9 mg N g^{-1} (Figure 2.2.13C). On the contrary, critical $[N]_{area}$ increased under e[CO_2] from 1.52 g N m^{-2} to 1.65 g N m^{-2} (Figure 2.2.13D).

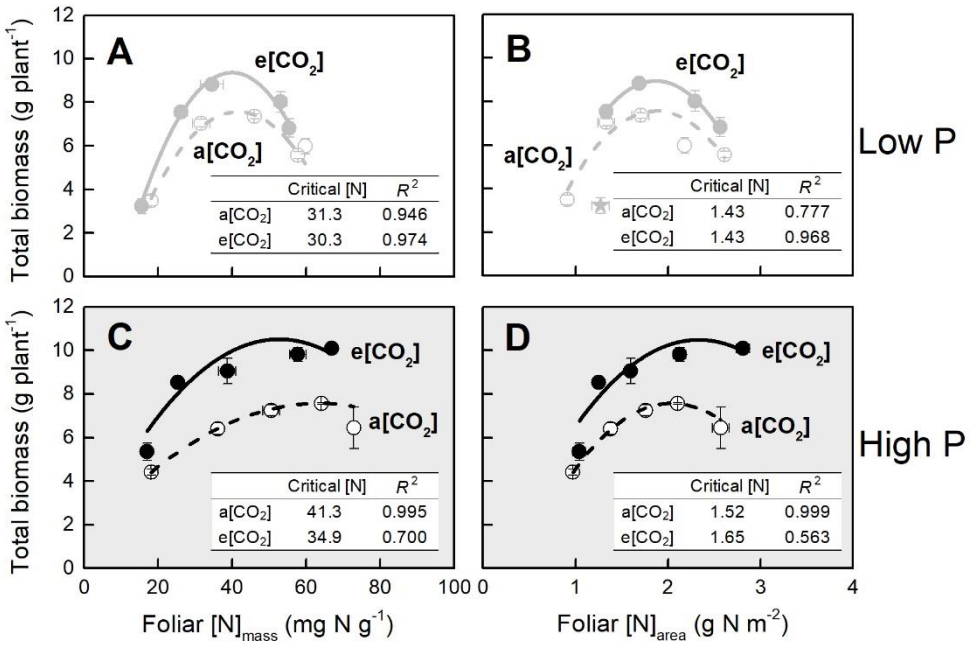

Figure 2.2.13 Relations between total biomass with foliar N concentration on mass basis ($[N]_{mass}$) under Low P (LP) **(A)** and High P (HP) **(C).** Relationships between total biomass with foliar N concentration on area basis ($[N]_{area}$) under LP **(B)** and HP **(D).** Critical [N] is defined as the minimum concentration of N required by the crop to reach 90% of maximum growth. Critical [N] and R^2 values for regressions are presented. Data in each plot are means ± S.E. (n = 4 biological replicates for each treatment). Regressions are as follows: **(A)** a[CO_2]: $y = -4.873 + 0.599x - 0.007x^2$, R^2 = 0.946; e[CO_2]: $y = -6.556 + 0.797x - 0.010x^2$, R^2 = 0.974. **(B)** a[CO_2]: $y = -6.252 + 14.771x - 3.945x^2$, R^2 = 0.777; e[CO_2]: $y = -7.453 + 17.554x - 4.698x^2$, R^2 = 0.968. **(C)** a[CO_2]: $y = 1.394 + 0.194x - 0.0015x^2$, R^2 = 0.995; e[CO_2]: $y = 1.298 + 0.350x - 0.0033x^2$, R^2 = 0.700. **(D)** a[CO_2]: $y = -3.979 + 11.322x - 2.772x^2$, R^2 = 0.999; e[CO_2]: $y = -1.703 + 10.460x - 2.244x^2$, R^2 = 0.563. The fitting line of e[CO_2] in (B) was fitted with four plots excluding the plot (star) shown in the figure, because fitting is unavailable when that plot is included.

2.2.3 Discussion

Which is suitable to assess critical nitrogen concentration, mass basis or area basis?

Generally, plants growing under e[CO_2] are expected to require a larger N supply to take full advantage of the CO_2-fertilization effect (Ainsworth and Long, 2005), because reduction in leaf N concentration caused by the dilution effect under e[CO_2] has significant effect on leaf photosynthesis and carbohydrate metabolic process (Li et al., 2016; Sanz-Sáez et al., 2010). In this study, considering the significant difference in LMA under different CO_2 conditions (Figure 2.2.6B and D), critical [N], as an index to evaluate N nutrition demand in plants, was assessed in two ways; based on mass ($[N]_{mass}$) and on area ($[N]_{area}$) (Figure 2.2.13). Similar to previous reports in cotton and wheat (Rogers et al., 1993), critical $[N]_{mass}$ was also decreased by e[CO_2] under HP in potato plants in this study (Figure 2.2.13C). However, critical $[N]_{area}$, which had not yet been examined in previous studies increased with e[CO_2] under HP (Figure 2.2.13D). The discrepancy may be due to more carbohydrates (e.g., starch) accumulating under e[CO_2] conditions (Figure 2.2.6C), which finally resulted in a higher LMA (Figure 2.2.6D). Both critical $[N]_{mass}$ and critical $[N]_{area}$ were similar between a[CO_2] and e[CO_2] regardless of different LMA under LP, implying an involvement of P nutritional status into the above discrepancy; however, the mechanism is unclear. Considering the light-capturing function of leaves in addition to such changes in LMA, it would be suitable to adopt per unit area ($[N]_{area}$) rather than mass basis ($[N]_{mass}$) as the critical [N] at leaf level, particularly when LMA is altered. Therefore, we have evaluated if N requirement was affected by CO_2 and P nutrition on basis of critical $[N]_{area}$ in addition to the minimum N supply for the maximum growth.

Is nitrogen requirement for the maximum growth affected by e[CO_2] with interaction of phosphorus nutrition?

Foliar critical $[N]_{area}$ was around 1.43 g N m^{-2} under both CO_2 conditions under LP (Figure 2.2.13B), but it increased from 1.52 to 1.65 g N m^{-2} under e[CO_2] under HP (Figure 2.2.13D). Consistent with critical $[N]_{area}$, the minimum N supply for the maximum plant growth was N0.4 under both CO_2 conditions under LP (Figure 2.2.4E), whereas it increased under e[CO_2] from N0.8 to N1.6 under HP (Figure 2.2.4J), especially in leaf biomass (Figure 2.2.4F). These results reveal that N requirement for the maximum growth increased under e[CO_2] unless P was deficient for the plant as mentioned below.

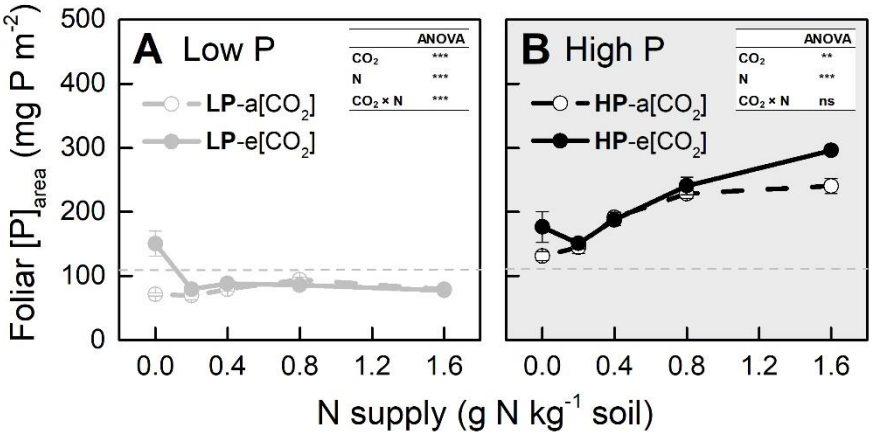

Figure 2.2.14 Foliar P concentration on area basis ($[P]_{area}$) of potato plants grown under a[CO_2] (395 ± 4 ppm) and e[CO_2] (802 ± 3 ppm) with different N supply rates (0, 0.2, 0.4, 0.8, and 1.6 g N kg^{-1} soil) at Low P (0.3 g P kg^{-1} soil) **(A)** and High P (3 g P kg^{-1} soil) **(B)**. Data in each plot are means ± S.E. (n = 4 biological replicates for each treatment). Statistical comparisons (two-way ANOVA) between CO_2 concentrations and N supply rates as well as their interaction (CO_2 × N) are presented.

In Chapter 2.1, foliar critical [P] in potato plants was around at 110 mg P m^{-2}. From Figure 2.2.14, foliar [P] under the LP condition was below this value except for N0 under e[CO_2] probably owing to the most severe N-limiting condition; however, foliar [P] under HP was above the value. The result clearly indicates P was sufficient for HP plants but not for LP ones, supporting our hypothesis that LP and HP treatments provided remarkable contrast that allows comparison between them. As for N status,

previous studies showed that foliar [N] in potato plants range from 20 to 90 mg N g^{-1} (Vos and van der Putten, 1998). In this study, foliar [N] ranged from 15 to 73 mg N g^{-1}, which covers a large range of N levels from N-deficient to N-sufficient. Based on these results, I have confirmed that the plants examined here had a wide range of N nutrition together with a high contrast of P nutrition.

As expected, e[CO_2] increased foliar N requirement for the maximum growth biomass only at HP (Figure 2.2.13). Contrary to that, LP decreased the requirement compared to HP for each CO_2 level (Figure 2.2.13), suggesting the strong growth limitation by P decreased N demand. Taub and Wang (2008) suggested two hypotheses to explain why $[N]_{mass}$ in plant tissue decreases under e[CO_2]; 1) dilution of N by increased C, 2) decrease in the specific uptake rates (per unit mass or length of root). As for the latter, they pointed out two factors; decreased N demand by shoots and decreased N supply from soil to root that is induced with transpiration-driven mass flow. In our results, N demand was higher in e[CO_2] unless P was deficient. Furthermore, e[CO_2] did not always suppress N uptake (Figure 2.2.12A and D) considering the similar leaf area excepting N1.6 at HP (Figure 2.2.9A and D) as well as plant transpiration (Figure 2.2.8A and C). These results would not likely support that e[CO_2] decreased either N demand by shoots or N supply from soil to root.

There was significant interaction between CO_2 and N supply on total plant biomass (P = 0.004 and 0.048 under LP and HP, respectively) (Figure 2.2.4E and J), indicating that additional N supply is required to sustain the positive effect of e[CO_2] perhaps via utilizing additional carbohydrates for the development of new sink organs (Uprety and Mahalaxmi, 2000). The NUE is a long-term indicator of availability of N utilization for C acquisition in plants (Wang et al., 2010). Grown under lower N condition would exacerbate the shortage of leaf N relative to C in plant (Stitt and Krapp, 1999), thus resulting in increased NUE (Figure 2.2.12C and F). Similarly, higher NUE under e[CO_2] also indicates relative N shortage comparing with that under a[CO_2], thus more N supply is required in a CO_2 enrichment condition.

The maximum total plant biomass was increased at e[CO_2] by 1.2- and 1.4-fold under LP and HP, respectively (Figure 2.2.13), which confirmed the CO_2-fertilization effect in potato plants. However, enhancement of the maximum total plant biomass by e[CO_2] was lower than in Trial P (1.5-fold). As Ainsworth et al. (2002) and Dong et al. (2017) pointed out, plant growth response to e[CO_2] is related to the growth stage. Thus, the difference in the increments of maximum plant biomass between Trial P and Trial N might be related to different growth stages. It is widely known that potato growth would be suppressed by nutrient deficiency, including N deficiency (Mokrani et al., 2018; Vos, 1997; Vos and van der Putten, 1998) and P deficiency (Alvarez-Sánchez et al., 1999; Fleisher et al., 2012). The increment of biomass by e[CO_2] was found to depend on both N and P supply in the current study (Figure 2.2.4). Additionally, starch was increased under both low N and low P supply as well as e[CO_2] (Figure 2.2.6A and C). This indicates photosynthesis may be inhibited by both nutrient deficiencies and e[CO_2], because over-accumulation of starch in leaves may damage internal organization of chloroplasts (Pritchard et al., 1997; Yelle et al., 1989) or hinder CO_2 diffusion in the chloroplasts (Jauregui et al., 2018; Makino and Mae, 1999; Sawada et al., 2001).

Is water requirement for the maximum growth affected by CO_2 with interaction of phosphorus nutrition?

Water use for the maximum plant biomass was unchanged by e[CO_2] under HP (N0.8 at a[CO_2] and N1.6 at e[CO_2]) but decreased under LP (N0.4 at the both [CO_2]) (Figure 2.2.8). Based on these results, it is likely that the water requirement for the maximum growth does not increase under the doubled CO_2 condition. This could be explained by the decreased g_s for e[CO_2] under both P supply rates (N0.4 vs. N0.4 in Figure 2.2.9B and N0.8 vs. N1.6 in Figure 2.2.9E) besides the similar leaf area (Figure 2.2.9A and D) at the corresponding N treatment achieving the maximum biomass.

Consistent with previous studies (Ainsworth and Rogers, 2007; Polley, 2002), e[CO_2] increased WUE significantly under each treatment in this study (Figure 2.2.8B and D). Moreover, both e[CO_2] and HP increased WUE independently from N supply (Table 2.2.1). The g_s under e[CO_2] was lower than that under a[CO_2] at each N supply rate, especially under HP (Figure 2.2.9B and E). However, stomatal density was unaffected by e[CO_2] (Figure 2.2.9C and F). In line with the statement by Finnan et al. (2005), decreases in g_s under e[CO_2] could be expected to improve WUE in potato plants.

As for the effects of P supply, WUE slightly increased under HP than LP at each N supply rate, except for a distinct increase at the N0 supply rate (Figure 2.2.8B and D). Like the effects of e[CO_2] on WUE, HP increased WUE by decreasing g_s in N-sufficient potato plants in Chapter 2.1. However, g_s was higher under HP compared to LP in this study (Figure 2.2.9B and E). Though the cause of the discrepancy of g_s in the two studies is unclear, it can be concluded that HP could increase WUE in potato plants.

Surprisingly, high N supply decreased WUE in potato plants with an increase in g_s as well as stomatal density (Figures 2.2.8 and 2.2.9), despite numerous literatures demonstrating increased WUE according to N supply (see review by Brueck, 2008). Wei et al. (2018) reported that plant WUE (total dry weight/ plant water use) was decreased under high N supply in tomato plants even with the higher intrinsic WUE (photosynthesis/ g_s) and instantaneous WUE (photosynthesis/ transpiration) compared to low N supply. This discrepancy indicates that intrinsic WUE or instantaneous WUE may not always reflect plant WUE, implying a difficulty to integrate intrinsic or instantaneous WUE measuring several mm^2 on a certain leaf at several minutes to a whole plant growing in several days. In this study, I speculated that the increase in biomass accumulation was smaller than the increase in water use during the growth period examined, thus lower WUE was observed at high N supply rate. This finding would be important for N fertilizer management at different growth stages in potato production.

2.2.4 Conclusions

The current study aimed at quantifying the growth response of potato plants to N supply under different CO_2 conditions and P supply rates to clarify how much N and water are required for maximum growth under each CO_2 condition and their dependencies on P supply. After carrying out trials, I found area based foliar critical [N] to be more suitable than mass based foliar critical [N] to evaluate plant N state. Based on this, I concluded that N requirement to obtain the maximum growth during the early growth stage in potato plants under e[CO_2] would be dependent on P supply; HP increased the N requirement, but LP would not affect it, probably owing to the limited growth by P. As for water requirement for the maximum growth, the doubled CO_2 condition would not likely increase it with enhanced WUE.

Chapter 2.3

Trial K

Plant growth and water economy of *Solanum tuberosum* in response to doubled CO_2: interaction between potassium and phosphorus

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2.3.1 Materials and methods

Experimental design and growing conditions

A pot experiment was carried out in GC with the same setting in Chapter 2.1. The actual conditions in the chambers were monitored every 5 min (Figure 2.3.1).

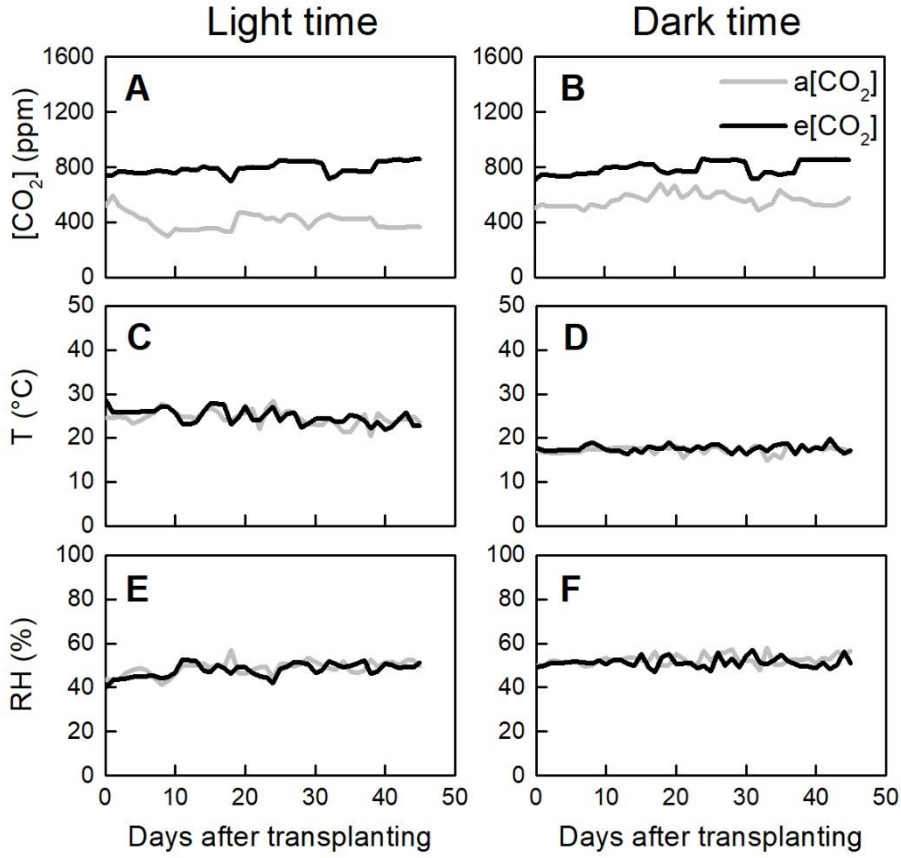

Figure 2.3.1 Actual conditions in growth chambers during growth period (46 days after transplanting). Actual CO_2 concentrations at light time **(A)** and dark time **(B)**. Actual temperature (T) at light time **(C)** and dark time **(D)**. Actual relatively humidity (RH) at light time **(E)** and dark time **(F)**.

Naturally sprouted potato tubers (cv. 'Irish Cobbler') were transplanted into 1-L pots (diameter, 11.3 cm; depth, 14 cm; one plant per pot) filled with 580 g of dry andosol. Before transplanting, N (0.4 g N kg^{-1} dry soil) was uniformly mixed with the soil in the form of urea (46.0% N). N topdressings (0.2 g N kg^{-1} dry soil) were supplied on the 13 and 18 DAT, respectively. Calcium superphosphate (17.5% P_2O_5) was uniformly mixed with the soil to control P supply rates at 0.1 (low P, LP) and 1.5 (high P, HP) g P kg^{-1} dry soil. Potassium chloride (60.0% K_2O) was uniformly mixed with

the soil to control K supply rates at 0, 0.1, 0.2, 0.4, or 0.8 g K_2O kg^{-1} of dry soil (hereafter, these treatments are designated as K0, K0.1, K0.2, K0.4, and K0.8, respectively). The soil water condition was maintained at approximately 80% (w/w). The experiment was organized following a factorial design (two CO_2 concentrations × two P supply rates × five K supply rates) with four biological replicates.

Stomatal conductance and stomatal density

On the day of harvest, the g_s of the youngest fully expanded leaf was measured on the adaxial surface between 8:00 to 12:00 in the morning using a leaf porometer (SC-1, Decagon Devices Inc., USA). Immediately after measurement of g_s, the same leaves were coated with nail polish; next, imprints were taken from each leaf and mounted on a glass microscope slide for stomatal density count in the same way in Chapter 2.1.

Harvest and sampling

All plants were harvested on the 46 DAT. Before harvest, the youngest fully expanded leaf was sampled from each plant for NSC quantification. After the leaf area was determined, the samples were dried for NSC analysis. At harvest, the remaining leaves, stems, roots, and tubers were separated and dried in an oven at 80 °C to a constant mass for dry weight determination. All samples were then ground to powder for P and K quantification.

Nutrient uptake, nutrient acquisition efficiency, and nutrient utilization efficiency

P and K uptake was defined as P and K content in total plant.

Nutrient acquisition efficiency and nutrient utilization efficiency were calculated following the report of Dobermann (2007):

P acquisition efficiency (PAE) (%) = P content in plant × 100% (g)/ P supply (g);

K acquisition efficiency (KAE) (%) = (K content in K-treated plant – K content in K0 plant) × 100% (g)/ K supply (g);

P utilization efficiency (PUE) (g mg^{-1}) = total plant biomass (g)/ total plant P content (mg);

K utilization efficiency (KUE) (g mg^{-1}) = total plant biomass (g)/ total plant K content (mg).

Effect size of e[CO_2]

Effect size (percent change at e[CO_2]) was calculated from the total plant biomass under a[CO_2] (a[CO_2] $_{biomass}$) and e[CO_2] (e[CO_2] $_{biomass}$), following the report of Ainsworth et al. (2002). Effect size (%) = (e[CO_2] $_{biomass}$ − a[CO_2] $_{biomass}$) × 100%/ a[CO_2] $_{biomass}$.

Statistical analysis

The experiment was organized in a factorial design with two CO_2 concentrations, two P supply rates, and five K supply rates with four biological replicates. Data are expressed as the mean ± standard error (S.E.) of four biological replicates. Data were analyzed using three-way ANOVA in SPSS 16.0 (SPSS Inc., Chicago, IL, USA) at the 0.05 level of probability (Table 2.3.1). Considering the significant interactions between CO_2 concentration and P supply rate from some of the main measured items, a two-way ANOVA was used to analyze the interaction between CO_2 concentration and K supply rate at each P supply rate (Table 2.3.1).

Table 2.3.1 Analysis of variance for the same variables is shown for the CO_2 concentration (CO_2), P supply (P), K supply (K), and interaction effects (CO_2 x P), (CO_2 x K), (P x K), (CO_2 x P x K).

	Biomass						Water use	WUE	g_s	Stomatal density	[P]			
	Leaf	Stem	Shoot	Root	Tuber	Total plant					Leaf	Stem	Root	Tuber
CO_2	0.135	0.869	0.628	0.025	< 0.001	< 0.001	0.001	< 0.001	0.215	0.915	< 0.001	0.093	0.139	< 0.001
P	< 0.001	< 0.001	< 0.001	0.147	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
K	< 0.001	0.157	0.035	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.005	< 0.001	< 0.001	< 0.001	0.129	< 0.001
CO_2 × P	0.010	0.575	0.145	0.898	< 0.001	0.004	< 0.001	< 0.001	0.600	0.588	< 0.001	< 0.001	0.088	< 0.001
CO_2 × K	0.049	0.846	0.400	0.391	0.245	0.143	0.065	0.098	0.902	0.740	0.142	0.249	0.675	0.315
P × K	< 0.001	0.439	0.012	0.132	< 0.001	< 0.001	< 0.001	< 0.001	0.789	0.064	0.095	< 0.001	0.885	0.004
CO_2 × P × K	0.832	0.950	0.905	0.867	0.126	0.160	0.667	0.035	0.670	0.400	0.597	0.161	0.724	0.202
Low P														
CO_2	0.004	0.671	0.088	0.172	0.012	0.001	0.451	< 0.001	0.674	0.654	0.032	0.109	0.592	0.488
K	0.078	0.023	0.050	< 0.001	0.593	0.351	< 0.001	< 0.001	0.213	0.089	< 0.001	< 0.001	0.008	0.006
CO_2 × K	0.160	0.448	0.251	0.639	0.176	0.134	0.235	0.505	0.948	0.924	0.559	0.538	0.621	0.733
High P														
CO_2	0.445	0.684	0.556	0.038	< 0.001	< 0.001	< 0.001	< 0.001	0.098	0.756	< 0.001	0.003	0.108	< 0.001
K	< 0.001	0.536	0.022	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.008	< 0.001	< 0.001	< 0.001	0.468	< 0.001
CO_2 × K	0.332	0.990	0.880	0.533	0.200	0.185	0.239	0.036	0.375	0.279	0.262	0.138	0.704	0.182

Continued to Table 2.3.1

	[K]												
	Leaf	Stem	Root	Tuber	P uptake	PAE	PUE	K uptake	KAE	KUE	Starch	Glucose	Sucrose
CO_2	< 0.001	< 0.001	0.880	< 0.001	0.121	0.017	0.002	0.259	0.009	< 0.001	< 0.001	< 0.001	0.001
P	< 0.001	< 0.001	0.297	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.614	0.061
K	< 0.001	< 0.001	0.213	< 0.001	0.006	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
CO_2 × P	0.033	0.121	0.674	0.033	0.059	0.016	0.230	< 0.001	0.004	< 0.001	0.035	0.578	0.347
CO_2 × K	0.018	0.524	0.914	0.018	0.624	0.282	0.540	0.005	0.422	0.160	0.812	0.015	0.102
P × K	< 0.001	< 0.001	0.916	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.554	< 0.001	< 0.001	< 0.001
CO_2 × P × K	0.078	0.304	0.606	0.078	0.265	0.250	0.302	0.469	0.230	0.467	0.446	< 0.001	0.043
Low P													
CO_2	< 0.001	0.032	0.822	0.553	0.019	0.019	0.283	0.002	< 0.001	0.003	< 0.001	0.012	0.006
K	< 0.001	< 0.001	0.140	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
CO_2 × K	0.017	0.387	0.908	0.817	0.277	0.277	0.487	0.022	0.229	0.081	0.048	0.081	0.607
High P													
CO_2	< 0.001	< 0.001	0.722	< 0.001	0.807	0.807	< 0.001	0.014	0.833	< 0.001	< 0.001	< 0.001	0.023
K	< 0.001	< 0.001	0.875	< 0.001	0.003	0.003	< 0.001	< 0.001	0.249	< 0.001	< 0.001	< 0.001	< 0.001
CO_2 × K	0.685	0.485	0.678	0.329	0.624	0.624	0.142	0.397	0.514	0.896	0.794	< 0.001	0.051

2.3.2 Results

Plant growth and biomass

Plant appearance at harvest is shown in Figure 2.3.2. There were no observed visual K deficiency symptoms. Based on the appearance of shoots, plant growth was apparently inhibited under LP, and it also seems to be slightly affected by CO_2 and K supply.

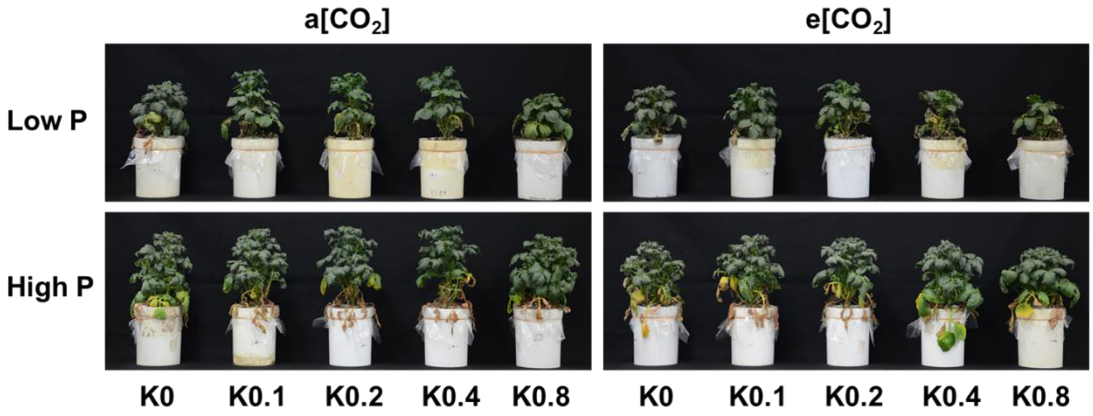

Figure 2.3.2 Appearance of potato plants at harvest (46 days after transplanting).

Total plant biomass was increased by e[CO_2] (Figure 2.3.3F) almost owing to an increase in tuber biomass (Figure 2.3.3E), even though there was a significant increase in root biomass ($P = 0.025$) (Figure 2.3.3D). There were no significant effects of e[CO_2] on biomass in leaves, stems, and shoots, except for a slight increase in leaf at K0.4 and K0.8 under LP (Figure 2.3.3A-C). Biomass of all organs, except for root, was increased in the HP group compared with the LP group. A strong interaction between CO_2 and P was observed in leaf, tuber, and total plant biomass. Tuber and total plant biomass increased with the increases in K supply under HP, but not under LP (Figure 2.3.3E and F). Leaf and shoot biomass were little affected by K supply, except for an increase at K0.8 under HP (Figure 2.3.3A and C). Stem and root biomass decreased with the increases in K supply at a certain range, and then remained unchanged (Figure 2.3.3B and D).

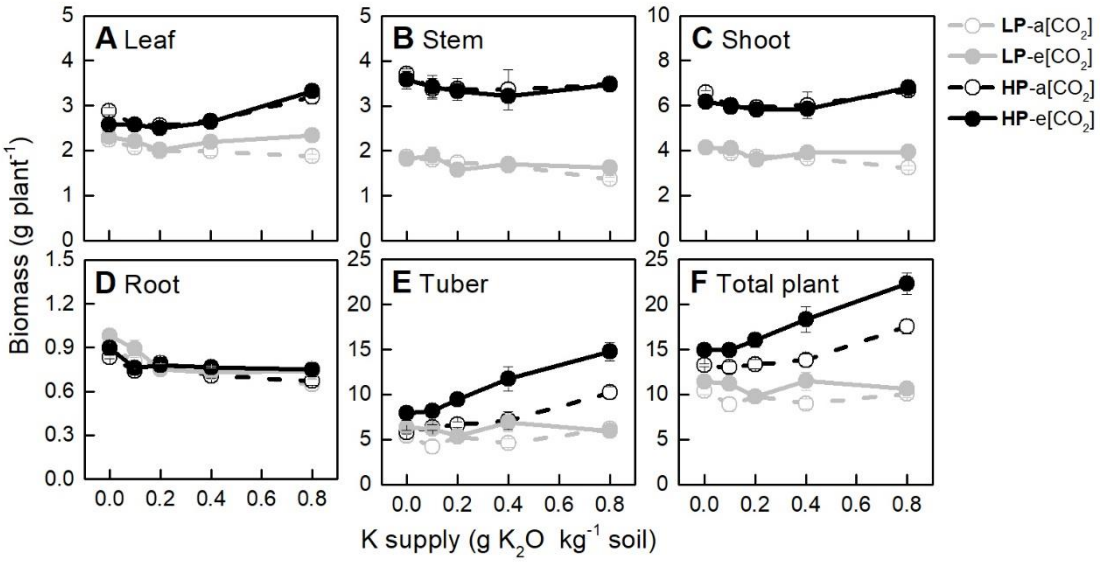

Figure 2.3.3 Biomass of several organs of potato plants grown under a[CO_2] (407 ± 9 ppm) and e[CO_2] (793 ± 6 ppm) at Low P (LP, 0.1 g P kg^{-1} soil) and High P (HP, 1.5 g P kg^{-1} soil) with different K supply rates (0, 0.1, 0.2, 0.4, and 0.8 g K_2O kg^{-1} soil). Data in each plot are means ± S.E. (n = 4 biological replicates for each treatment). **(A)** Leaf biomass; **(B)** stem biomass; **(C)** shoot biomass; **(D)** root biomass; **(E)** tuber biomass; and **(F)** total plant biomass.

Comparing with a[CO_2], average percent changes of total biomass at e[CO_2] were approximately 14% and 22% under LP and HP, respectively (Figure 2.3.4). There was no significant relationship between effect size and K supply under LP; however, the effect size increased with the increases in K supply under HP and reached a maximum value at K0.4.

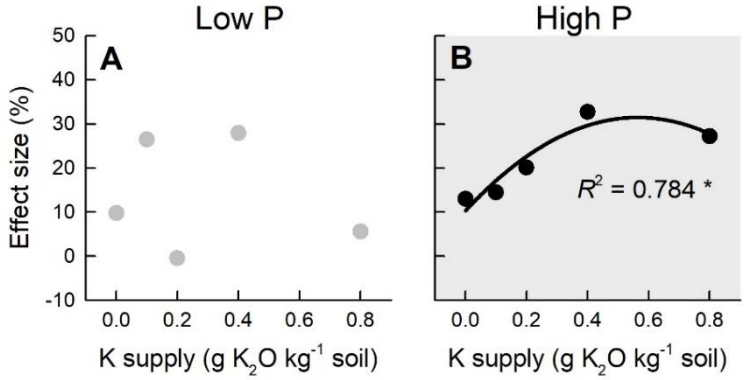

Figure 2.3.4 Effect size (percent change at e[CO_2]) of potato plants grown under a[CO_2] (407 ± 9 ppm) and e[CO_2] (793 ± 6 ppm) at Low P (LP, 0.1 g P kg^{-1} soil) and High P (HP, 1.5 g P kg^{-1} soil) with different K supply rates (0, 0.1, 0.2, 0.4, and 0.8 g K_2O kg^{-1} soil). **(A)** Effect size of e[CO_2] under LP, and **(B)** effect size of e[CO_2] under HP. Effect size (%) = (e[CO_2] $_{biomass}$ − a[CO_2] $_{biomass}$) × 100%/ a[CO_2] $_{biomass}$. No significant relation between effect size and K supply under LP was observed. Regression under HP is as follows: $y = -66.2x^2 + 74.8x + 10.2$, $R^2 = 0.784$, $P = 0.018$.

Tuber proportion was increased by e[CO_2] increased at the expense of dry matter of leaf and stem, especially under HP (Figure 2.3.5). The average increase in tuber proportion by e[CO_2] was larger under HP (9%) than under LP (3%). Biomass allocation to tuber was little affected by K supply under LP; however, it increased with the increases in K supply under HP.

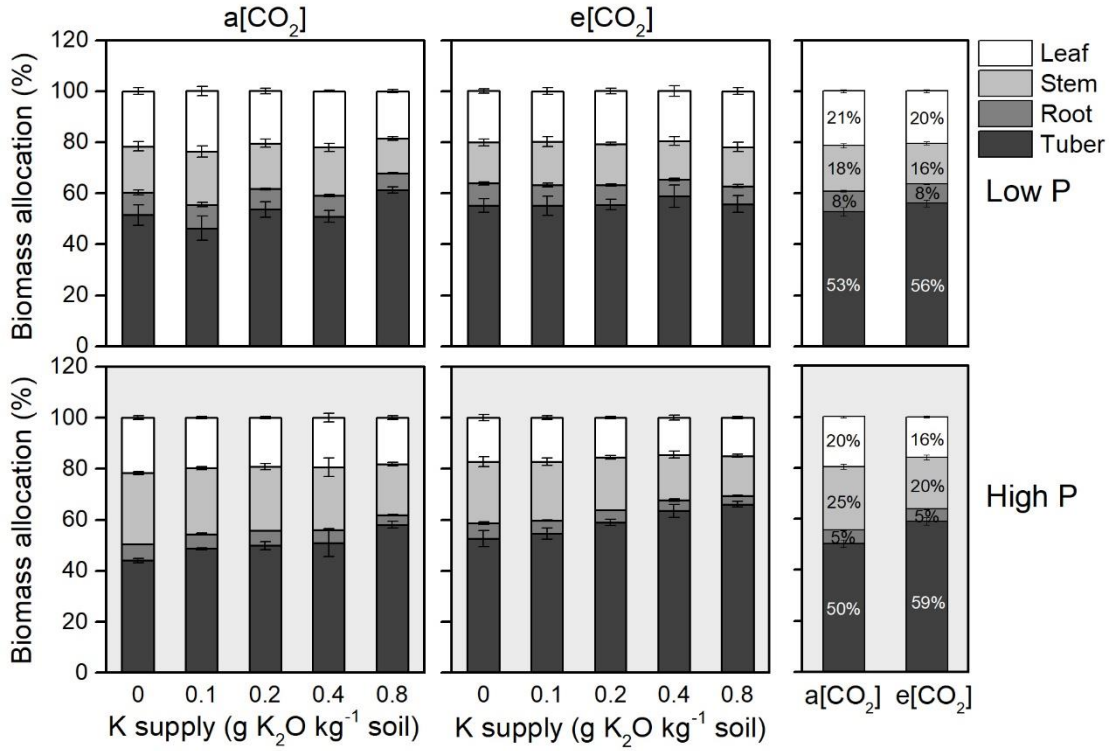

Figure 2.3.5 Biomass partitioning in organs of potato plants grown under a[CO_2] (407 ± 9 ppm) and e[CO_2] (793 ± 6 ppm) at Low P (LP, 0.1 g P kg^{-1} soil) and High P (HP, 1.5 g P kg^{-1} soil) with different K supply rates (0, 0.1, 0.2, 0.4, and 0.8 g K_2O kg^{-1} soil). Data in each plot are means ± S.E. (n = 4 biological replicates for each treatment).

Water use and water-use efficiency

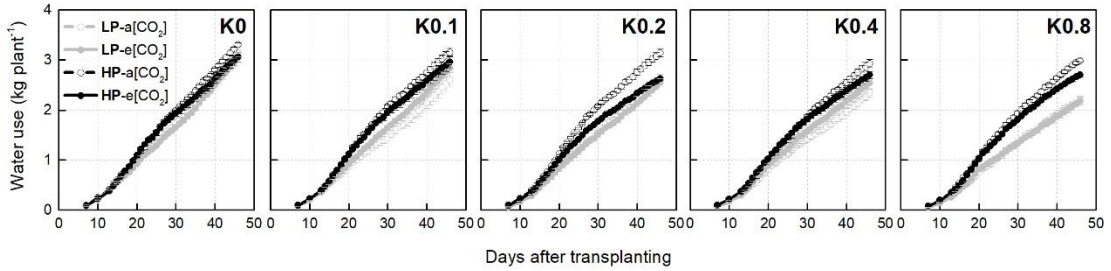

Figure 2.3.6 Water use by potato plants grown under a[CO_2] (407 ± 9 ppm) and e[CO_2] (793 ± 6 ppm) at Low P (LP, 0.1 g P kg^{-1} soil) and High P (HP, 1.5 g P kg^{-1} soil) with different K supply rates (0, 0.1, 0.2, 0.4, and 0.8 g K_2O kg^{-1} soil). Data in each plot are means ± S.E. (n = 4 biological replicates for each treatment).

I monitored time course changes in cumulative transpiration as water use in potato plants (Figure 2.3.6).

Total water use during the growth period was reduced by e[CO_2] only under HP (P < 0.001), but not under LP (P = 0.451) (Figure 2.3.7A). Water use gradually decreased with the increases in K supply under LP; however, it reached a minimum and then remained unchanged under HP.

Significant increases in WUE by e[CO_2] were observed under both P supply rates, especially under HP (Figure 2.3.7B). WUE under HP was remarkably higher than that under LP. With the increases in K supply, WUE increased under both P supply rates.

Both stomatal density and g_s were unaffected by e[CO_2] under both P supply (Figure 2.3.7C and D). Compared with LP, HP decreased g_s but increased stomatal density. With the increases in K supply, g_s increased gradually until K0.2, and then decreased; however, it was not significant under LP (P = 0.213) (Figure 2.3.7C). Stomatal density decreased with an increase in K supply under HP (P < 0.001) but was little affected under LP (P = 0.089) (Figure 2.3.7D).

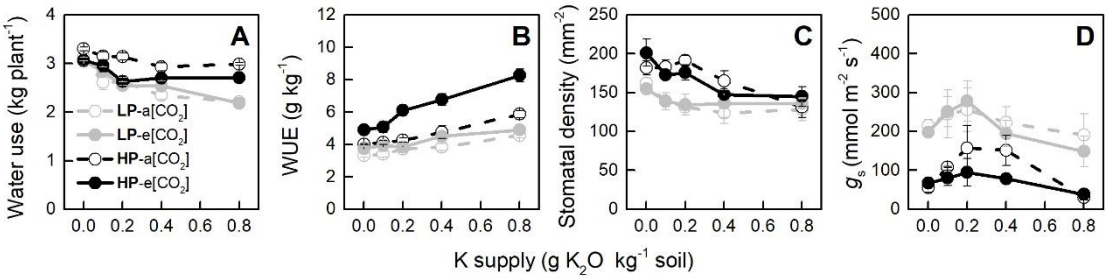

Figure 2.3.7 (A) Water use, **(B)** water-use efficiency (WUE), **(C)** stomatal density, and **(D)** stomatal conductance (g_s) of potato plants grown under a[CO_2] (407 ± 9 ppm) and e[CO_2] (793 ± 6 ppm) at Low P (LP, 0.1 g P kg^{-1} soil) and High P (HP, 1.5 g P kg^{-1} soil) with different K supply rates (0, 0.1, 0.2, 0.4, and 0.8 g K_2O kg^{-1} soil). Data in each plot are means ± S.E. (n = 4 biological replicates for each treatment).

Phosphorus and potassium status

[P] in leaves, stems, roots, and tubers was clearly higher in plants supplied with HP than those supplied with LP at each K supply rate (Figure 2.3.8). Leaf [P] was decreased by e[CO_2] under both P supply rates. [P] in stems and tubers was little affected by e[CO_2] under LP, but was decreased by e[CO_2] under HP (Figure 2.3.8B and D). Root [P] was unaffected by e[CO_2] under both LP and HP conditions (Figure 2.3.8C). Additionally, [P] in these organs, except for the root, decreased with the increases in K supply.

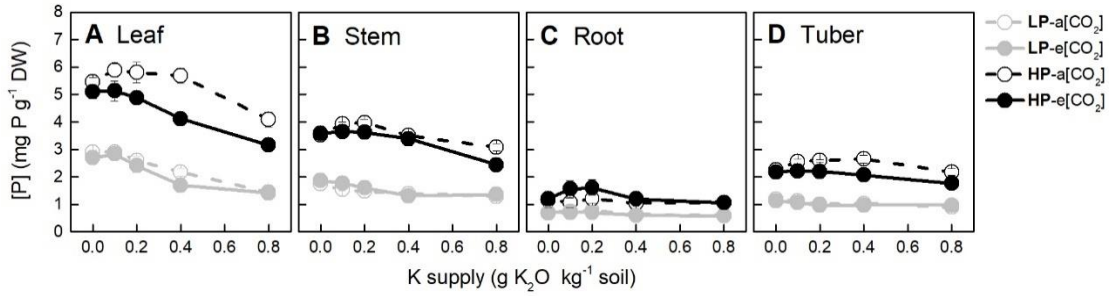

Figure 2.3.8 P concentration ([P]) of several organs of potato plants grown under a[CO_2] (407 ± 9 ppm) and e[CO_2] (793 ± 6 ppm) at Low P (LP, 0.1 g P kg^{-1} soil) and High P (HP, 1.5 g P kg^{-1} soil) with different K supply rates (0, 0.1, 0.2, 0.4, and 0.8 g K_2O kg^{-1} soil). Data in each plot are means ± S.E. (n = 4 biological replicates for each treatment). **(A)** Leaf [P]; **(B)** stem [P]; **(C)** root [P]; and **(D)** tuber [P].

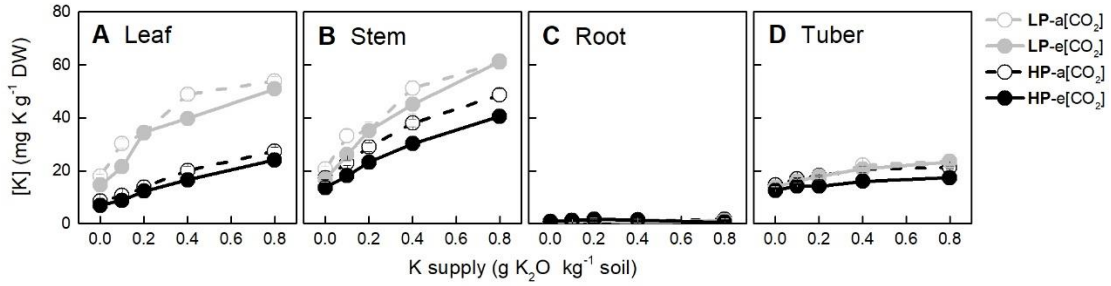

Figure 2.3.9 K concentration ([K]) of several organs of potato plants grown under a[CO_2] (407 ± 9 ppm) and e[CO_2] (793 ± 6 ppm) at Low P (LP, 0.1 g P kg^{-1} soil) and High P (HP, 1.5 g P kg^{-1} soil) with different K supply rates (0, 0.1, 0.2, 0.4, and 0.8 g K_2O kg^{-1} soil). Data in each plot are means ± S.E. (n = 4 biological replicates for each treatment). **(A)** Leaf [K]; **(B)** stem [K]; **(C)** root [K]; and **(D)** tuber [K].

[K] in leaves, stems, and tubers increased with an increase in K supply (Figure 2.3.9). Compared with LP, HP decreased [K] in all organs except for the root. Moreover,

e[CO_2] decreased [K] in all organs except for the root under HP. Under LP, [K] was decreased by e[CO_2] in leaves and stems only at certain K supply rates (K0.1 and K0.4).

P uptake was unaffected by e[CO_2] under HP (P = 0.807), but slightly increased under LP (P = 0.019) according to the results of two-way ANOVA analysis (Figure 2.3.10A). Accordingly, PAE was unaffected by e[CO_2] under HP, but was a little higher under e[CO_2] than a[CO_2] under LP (Figure 2.3.10B). Both P uptake and PAE decreased with the increases in K supply only under LP, but slightly increased with the increases in K supply under HP. As for PUE, e[CO_2] increased them at each K supply only under HP. PUE increased with the increases in K supply under both P supply rates (Figure 2.3.10C). Both PAE and PUE were lower under HP than under LP at each K supply rate.

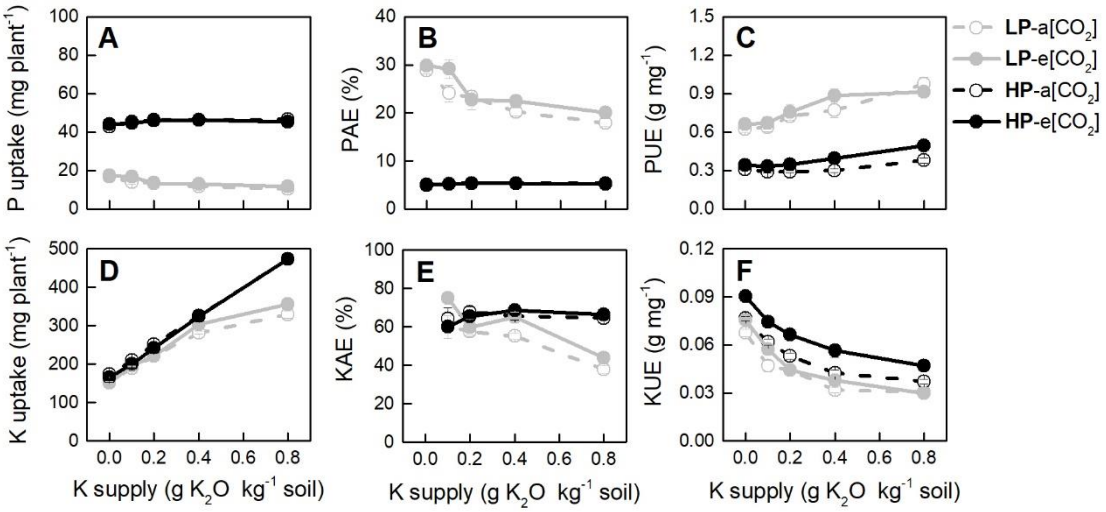

Figure 2.3.10 (A) P uptake, **(B)** P acquisition efficiency (PAE), **(C)** P utilization efficiency (PUE), **(D)** K uptake, **(E)** K acquisition efficiency (KAE), and **(F)** K utilization efficiency (KUE) of potato plants grown under a[CO_2] (407 ± 9 ppm) and e[CO_2] (793 ± 6 ppm) at Low P (LP, 0.1 g P kg^{-1} soil) and High P (HP, 1.5 g P kg^{-1} soil) with different K supply rates (0, 0.1, 0.2, 0.4, and 0.8 g K_2O kg^{-1} soil). Data in each plot are means ± S.E. (n = 4 biological replicates for each treatment). PAE (%) = P content in plant × 100% (g)/ P supply (g). PUE (g mg^{-1}) = Total plant biomass (g)/ Total plant P content (mg). KAE (%) = (K content in K_treated plant – K content in K0 plant) × 100% (g)/ K supply (g). KUE (g mg^{-1}) = Total plant biomass (g)/ Total plant K content (mg).

K uptake increased with the increases in K supply under both P supply rates (Figure 2.3.10D). K uptake was increased by e[CO_2] under LP at K0.4 and K0.8, but slightly decreased under HP (P = 0.014). K uptake was higher under HP than under LP,

especially at K0.8 (Figure 2.3.10D). KAE was increased by e[CO_2] only under LP (Figure 2.3.10E). KAE and KUE were higher under HP than under LP (Figure 2.3.10E and F). With the increases in K supply, KAE and KUE decreased under LP. Under HP, KAE was unaffected by e[CO_2] and K supply; however, KUE was increased by e[CO_2] and decreased with the increases in K supply.

Non-structural carbohydrates in the youngest fully expanded leaves

The e[CO_2] increased the starch concentration of the youngest fully expanded leaf compared with that of the a[CO_2] under both P supply rates (Figure 2.3.11A). Leaf starch concentration was higher under HP than under LP at e[CO_2]. For K supply, leaf starch continued to increase with the increases in K supply under e[CO_2], except for K0 under LP. Leaf starch under a[CO_2] was little affected by K supply, except for the remarkable increase at K0.8 under HP.

Glucose and sucrose concentrations were little affected by P supply (Figure 2.3.11B and C). The effect of e[CO_2] on glucose and sucrose was complicated at each K supply, but showed an increasing trend. With the increases in K supply, both glucose and sucrose decreased to the minimum and then remained unchanged, except at K0.8 under HP.

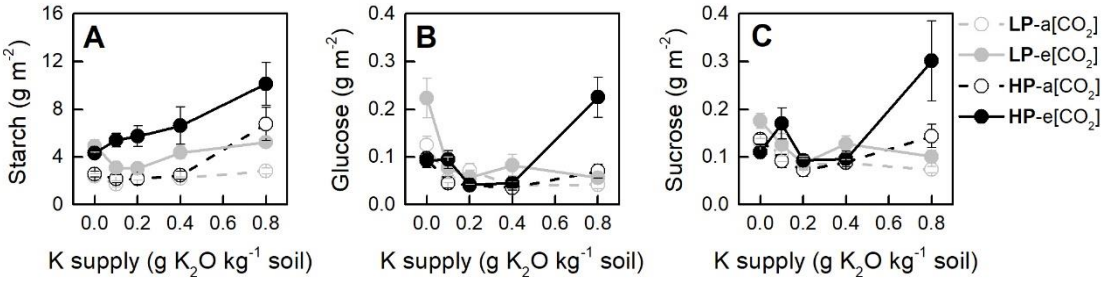

Figure 2.3.11 (A) Starch concentration, **(B)** glucose concentration, and **(C)** sucrose concentration in the youngest fully expanded leaf of potato plants grown under a[CO_2] (407 ± 9 ppm) and e[CO_2] (793 ± 6 ppm) at Low P (LP, 0.1 g P kg^{-1} soil) and High P (HP, 1.5 g P kg^{-1}soil) with different K supply rates (0, 0.1, 0.2, 0.4, and 0.8 g K_2O kg^{-1} soil). Data in each plot are means ± S.E. (n = 4 biological replicates for each treatment).

2.3.3 Discussion

This experimental setup was suitable to impose both P and K deficiency in potato plants. The results of leaf tissue analysis could be used as an indicator of nutrient adequacy status in plants. Leaf [P] ranging from 3 to 5 mg P g^{-1} DW are thought to be adequate for crop growth (Marschner, 1995). In this study, leaf [P] in HP was above 3 mg P g^{-1} DW, but below that value in LP (Figure 2.3.8A). In addition, this study recorded a wide range of [K], ranging from 6.9. to 53.8 mg K g^{-1} DW in leaf (Figure 2.3.9A). The critical nutrient ranges for K at 95 and 100% of the maximum yield were 50.4 to 51.5, 41.7 to 45.5, and 36.8 to 38.9 mg K g^{-1} DW for the fourth leaf at 30 days, 45 days, and 60 days after planting (Sharma and Arora, 1989). Despite of the fact that the range of leaf K was a little narrow under HP (6.9-27.4 mg K g^{-1} DW), K supply promoted plant growth remarkably under a P-sufficiency condition (Figure 2.3.3F). It needs to be pointed out that this experiment was conducted at a small scale (plants grew in 1-L pot), which may inhibit CO_2-fertilization effect, because root volume per plant (pot size) and sink capacity (tuber) are important factors affecting photosynthesis (Ainsworth et al., 2002; Hasegawa et al., 2013). Despite of this, significant interaction between CO_2 and nutrient supply on biomass accumulation and water use was observed in this study, which could make significant contribution to potato production in an increasing CO_2 environment in future.

K supply promoted plant growth in potato plants under e[CO_2] by enhancing tuber growth

K enhanced plant growth in potato plants primarily by promoting tuber growth (Figure 2.3.3). From Figure 2.3.5, tuber proportion increased with the increases in K supply under HP. K can establish an osmotic potential within the phloem, which is needed to translocate sucrose from source to sink organs (Cakmak et al., 1994). In this study, accumulation of sucrose reduced with K supply (except at K0.8) (Figure 2.3.11C), indicating that the formation of potato tubers was promoted by K supply.

Accumulation of starch in leaves is thought to be unfavorable for photosynthesis, because the down-regulation of photosynthesis under e[CO_2] is often attributable to insufficient capacity of sink to use or store carbohydrates (Lemonnier and Ainsworth, 2018). In this study, leaf starch was increased by e[CO_2] at each K supply rate (Figure 2.3.11A), probably indicating a certain degree of down-regulation of photosynthesis under e[CO_2]. P deficiency has been reported to cause starch accumulation in leaves by affecting photosynthetic electron transport chain (Carstensen et al., 2018); however, leaf starch was higher under HP than under LP in this study (Figure 2.3.11A). The role of starch in plant growth and development is related to ever-changing energy requirements (MacNeill et al., 2017). Starch accumulation in vegetative tissues has also been reported to be important for crop production because of carbon remobilization from transitory starch to yield (Schlosser et al., 2012). K enhanced starch because K is the most efficient monovalent cation in the activation of starch synthase, and thus responsible for starch synthesis (Hawker et al., 1974), corroborating the result of my study, in which starch increased with an increase in K supply under e[CO_2] (Figure 2.3.11A).

CO_2-fertilization effect depends on both phosphorus and potassium supply

Similar to many previous studies on C_3 plants (see review by Kimball, 2016), growth promotion by e[CO_2] was observed in this study (Figure 2.3.3). However, percent change at e[CO_2] was found to be dependent on both P and K supply. Plant growth was clearly inhibited under LP compared to it under HP (Figure 2.3.4). That P deficiency led reduction in biomass across CO_2 levels was also reported in cotton (Singh et al., 2013a). For K supply, tuber and total plant biomass were little affected by e[CO_2] under LP owing to P deficiency; however, enhancement by e[CO_2] increased with the increases in K supply under HP (Figure 2.3.3E and F). Percent change of total plant biomass at e[CO_2] showed that CO_2-fertilization effect was little affected by K supply under LP, but increased with the increases in K supply under HP (Figure 2.3.4).

A significant decrease in macronutrient elements, including P and K, by e[CO_2] was reported by Taub & Wang (2008). Since the requirement for mineral elements for key metabolic processes is increased by enhanced carbon fixation, and to fully benefit from elevated CO_2 levels, sufficient and balanced nutrient supply must be met for crop production. In this study, both P and K uptake were little affected by e[CO_2] (Figure 2.3.10A and D), though two-way ANOVA suggested a significant increase under LP (Table 2.3.1). Accordingly, PAE and KAE were slightly increased by e[CO_2] under LP (Figure 2.3.10B and E). PAE was unaffected by e[CO_2] under HP in this trial, which is inconsistent with previous study in Trial P where PAE was increased by e[CO_2], probably owing to the different growth stages examined. Miglietta et al. (1998) found that e[CO_2] advanced the flowering date and accelerated canopy senescence in potato crops, and may also affect nutrient uptake and acquisition efficiency. It was always thought that uptake of soluble nutrients (e.g., N and K) is related to transpiration flow; however, K uptake seems to be little related to it because water use was decreased by e[CO_2] under HP and unaffected under LP (Figures 2.3.7A and 2.3.10D). Both plant [P] and [K] (1/PUE and 1/KUE) were decreased by e[CO_2], and could be explained by the dilution effect owing to an increase in biomass.

Economical water use under e[CO_2] interacting with both phosphorus and potassium nutrients

There was a significant interaction between CO_2, P, and K on WUE according to the results of the three-way ANOVA analysis (P = 0.035). WUE was remarkably increased by e[CO_2], especially under HP conditions (Figure 2.3.7B). The increase in WUE by e[CO_2] was due to both increased biomass and decreased water use (Figures 2.3.3F and 2.3.7A). Most previous studies have suggested that e[CO_2] enhances WUE by promoting photosynthesis capacity and decreasing g_s (see review by Polley, 2002). Ainsworth & Rogers (2007) and Bettarini et al. (1998) reported that stomatal density was unaffected by e[CO_2] in most species. Similarly, stomatal density in the examined potato cultivar (Irish Cobbler) responded little to e[CO_2] in this study (Figure 2.3.7C),

which was also reported in Trials P and N. In this study, the effect of e[CO_2] on g_s was not significantly different under both P supply (P = 0.674 and 0.098 for LP and HP, respectively), though it was reduced by e[CO_2] at the certain K supply (K 0.2 and 0.4) under HP (Figure 2.3.7D). Pearson and Brooks (1995) reported that transpiration rate was lower under e[CO_2], but the values declined rapidly with leaf age and then showed no difference between a[CO_2]and e[CO_2]. In this study, g_s was similar before harvest between a[CO_2] and e[CO_2] probably owing to the late growth stage when photosynthetic acclimation occurs.

Total water use during growth period decreased with increasing K supply (Figure 2.3.7A). K enhanced plant growth without additional water demand owing to high WUE (Figure 2.3.7B). Furthermore, I also observed a low stomatal density under high K supply (Figure 2.3.7C) and likely contributed to the water economy in potato plants. Although the change in g_s was complicated with the increases in K supply, the highest K supply decreased g_s compared to that without K supply. Consistent with the results in Trial P, WUE was higher under HP than under LP (Figure 2.3.7B), and may be partly due to the decreased g_s by P supply (Figure 2.3.7D). Consistent with the report on cowpea by Sekiya and Yano (2008), HP increased stomatal density in this study (Figure 2.3.7C). Despite less response of stomatal density in potato to P supply reported by Sun et al. (2014), they found greater stomatal density associates with higher WUE. That is because leaf stomatal density associates with carbon isotope discrimination ($\Delta^{13}C$) (Sekiya and Yano, 2008; Sun et al., 2014; Yan et al., 2012), and $\Delta^{13}C$ is thought a valid proxy to estimate long-term whole-plant WUE (Adiredjo et al., 2014; Farguhar et al., 1989).

2.3.4 Conclusions

This study aimed to investigate the growth response of potato plants to e[CO_2] under different K and P supply rates. After conducting trials, I found that there was a significant interaction between K supply and P supply, but not between CO_2 and K (Table 2.3.1). K could remarkably enhance plant biomass accumulation under e[CO_2] by promoting tuber formation. The maximum biomass was increased by approximately 1.3-fold under e[CO_2] in this study; however, the CO_2-fertilization effect was dependent on both P and K supply. K supply resulted to less biomass accumulation in plants with P deficiency, indicating that a balanced nutrient status is crucial for crop production. Additionally, WUE was increased by e[CO_2] and P and K supply. Both CO_2 enrichment and K supply increased WUE by stimulating biomass accumulation and reducing water consumption.

Chapter 3

Potato in response to elevated CO_2 under various nutrient status: effects of developmental stages and chambers

3.1 Introduction

Potato is the most important non-grain crop in the world (Raymundo et al., 2018). How nutrient supply affects CO_2 effect on potato growth have been little examined except for Fleisher et al. (2012, 2013b). In Chapter 2, growth responses of potato plants to e[CO_2] was significantly affected by N and P. However, these experiments were conducted in GC during relatively short-term growth period and at small scale. Previous study shows that CO_2 effects varied under different developmental stages and CO_2 enrichment facilities (Ainsworth et al., 2002). As Körner et al. (1995) indicated, the experiment is more realistic, the results will be more valuable to provide a basis for modeling the future of the biosphere. For crop production, study at large scale and during long experimental duration is more valuable. Thus, further study is needed to verify CO_2 effects on potato growth in long-term exposure to e[CO_2] and at large scale.

In this chapter, four experiments were conducted, designated as Trial 1, Trial 2, Trial 3, and Trial 4, respectively. Trial 1 was conducted in GC covering the entire growth period to investigate effects of long-term exposure to e[CO_2] on potato growth across N- and P-deficiency conditions. To further verify whether CO_2 effects can be maintained at relatively large scale, Trials 2, 3 and 4 were conducted in OTC under various nutrient status and at different developmental stages in the same potato cultivar (Irish Cobbler) as in Trial 1. Since plant growth varied under different conditions, effect size, defined as percent change at e[CO_2], was used in this chapter to estimate CO_2 effects under various conditions. The objectives in this chapter were 1) to investigate CO_2 effects on potato growth until entire maturity, 2) to examine CO_2 effects on potato growth in OTC at different developmental stages, 3) to clarify how nutrient supply affects CO_2 effects on potato growth.

3.2 Materials and methods

Trial 1

A pot experiment was carried out in GC with the same setting in Chapter 2.1. The actual conditions in the chambers were monitored every 5 min (Figure S3.2).

Naturally sprouted potato tubers (cv. 'Irish Cobbler') were transplanted into 1-L pots (diameter, 11.3 cm; depth, 14 cm; one plant per pot) filled with 0.58 kg of dry andosol. Before transplanting, K (0.4 g K_2O kg^{-1} soil) was uniformly mixed with the soil in the form of potassium chloride (60.0% K_2O) in all pots. N was uniformly mixed with the soil in the form of urea (46.0% N) to control N at two supply levels: low N (0.1 g N kg^{-1} dry soil) and high N (0.8 g N kg^{-1} dry soil). For low N, all N fertilizer was amended into soil before transplanting. For high N supply, 0.4 g N kg^{-1} dry soil was uniformly mixed with the soil before transplanting, and then, N topdressing (0.2 g N kg^{-1} dry soil) were supplied on the 13 and 16 DAT, respectively. P was uniformly mixed with the soil in the form of calcium superphosphate (17.5% P_2O_5) before transplanting to control P at two supply levels: low P (0.1 g P kg^{-1} dry soil) and high P (1.5 g N kg^{-1} dry soil). There were three treatments: Control (high N and high P); Low N (LN, low N and high P); Low P (LP, high N and low P). Four biological replicates for each treatment. Soil water content was kept approximately at 80% (w/w).

Plants were harvested until entire senescence. Plants with different nutrient supply had different lifespans. Thus, plants were harvested on the 50, 85, and 94 DAT for Control, LN, and LP, respectively.

Trial 2

A pot experiment was carried out in OTC located in Nagoya University, in 2019 from the 11th, April to the 8th, June. The square type OTC (2 m × 2 m) is assembled by galvanized iron pipe and installed in experimental field (Figure S3.1). It is enclosed by plastic sheet (polyvinyl chloride) for the regulation of CO_2 concentration. It has an open

top for the maintenance of natural conditions, such as sunlight, temperature, and relative humidity. CO_2 concentration for a[CO_2] was not applied with CO_2, thus kept at ambient CO_2 level (approximately at 400 ppm). The CO_2 concentrations were controlled at approximately 800 ppm for e[CO_2] only at day time (6:00 – 18:00). During the night time (18:00 – 6:00), CO_2 were not applied. CO_2 gas were supplied from the 15 DAT due to equipment malfunction. The actual conditions in the chambers were monitored every 5 min (Figure S3.3).

Naturally sprouted potato tubers (cv. 'Irish Cobbler') were transplanted into 7.4-L pots (diameter, 22 cm; depth, 25 cm; one plant per pot) filled with 3.2 kg of dry andosol. Before transplanting, N, P and K were uniformly mixed with the soil before transplanting in the form of urea (46.0% N), calcium superphosphate (17.5% P_2O_5) and potassium chloride (60.0% K_2O). LN and HN were set as 0.3 and 3 g N kg^{-1} soil, respectively. LP and HP were set as 0.3 and 3 g P kg^{-1} soil, respectively. Low K (LK) and High K (HK) were set as 0.3 and 3 g K_2O kg^{-1} soil, respectively. Soil water conditions were controlled at well-water condition. The experiment was organized following a factorial design (two CO_2 concentrations × two N supply rates × two P supply rates × two K supply rates) with five biological replicates.

Plants were harvested on the 58 DAT.

Trial 3

A pot experiment was carried out in OTC, the same with Trial 2, in 2020 from the 17th, October to the 27th, December. The actual conditions in the chambers were monitored every 5 min (Figure S3.4).

Naturally sprouted potato tubers (cv. 'Irish Cobbler') were transplanted into 7.4-L pots (diameter, 22 cm; depth, 25 cm; one plant per pot) filled with 3.2 kg of dry andosol. Before transplanting, calcium superphosphate (17.5% P_2O_5) was uniformly mixed with the soil to control P levels at 0.3 (LP) and 3 (HP) g P kg^{-1} dry soil. N (0.5 g

N kg^{-1} dry soil) and K (1.2 g K_2O kg^{-1} dry soil) were uniformly mixed with the soil in the form of potassium chloride (60% K_2O), urea (46.0% N), and calcium superphosphate (17.5% P_2O_5), respectively. As topdressing, 0.25 g N kg^{-1} dry soil and 0.6 g K_2O kg^{-1} dry soil dissolved in tap water was added to each pot at 12 DAT. At 31 DAT, 0.25 g N kg^{-1} dry soil was applied again. Three times for sampling were done on the 26, 48, and 71 DAT, respectively. The experiment was organized following a factorial design (three stages × two CO_2 concentrations × two P supply) with four biological replicates.

Plants were sampled on the 26, 48, and 71 DAT, respectively. Before harvest, SPAD of the oldest leaf blade on each node was measured.

Trial 4

A pot experiment was carried out in OTC, the same with Trial 2, in 2018 from the 10th, April to the 4th, June. The actual conditions in the chambers were monitored every 10 min (Figure S3.5).

Naturally sprouted potato tubers (cv. 'Irish Cobbler') were transplanted into the pots (length, 60 cm; width, 17; depth, 18 cm; one plant per pot) filled with 5.8 kg of dry andosol. Before transplanting, N (0.6 g N kg^{-1} dry soil) and K (1.2 g K_2O kg^{-1} dry soil) were uniformly mixed with the soil in the form of ammonium sulfate (21.0% N) and potassium chloride (60.0% K_2O). Calcium superphosphate (17.5% P_2O_5) were uniformly mixed with the soil to control P levels at 0.3 (LP) and 3 (HP) g P kg^{-1} dry soil. Soil water condition were controlled at well-water condition. The experiment was organized following a factorial design (two CO_2 concentrations × two P supply rates) with eight biological replicates.

The youngest fully expanded leaves were sampled for NSC quantification on the 48 DAT. Plants were harvested on the 55 DAT.

Statistical analyses

Data were analyzed in SPSS 16.0 (SPSS Inc., Chicago, IL., USA). Statistical analysis was carried out separately for different trials. *t*-test was performed at the 0.05 probability level to access CO_2 effects under each nutrient status in Trial 1. Multi-factor ANOVA was performed at the 0.05 probability level in Trials 2 (Table S1), 3 (Table S2) and 4, respectively. Effect size (%) = $(e[CO_2]_{biomass} - a[CO_2]_{biomass}) \times 100\%/a[CO_2]_{biomass}$ (Ainsworth et al., 2002).

Table 3.1 Summary of actual growth conditions in chambers during growth period.

		Chamber type	Time	CO_2 concentration (ppm)			Temperature (℃)			Relative humidity (%)		
				a[CO_2]	e[CO_2]		a[CO_2]	e[CO_2]		a[CO_2]	e[CO_2]	
Day time	Trial 1	GC	5:00-19:00	387.9 ± 3.8	781.9 ± 2.5	(***)	26.6 ± 0.1	26.2 ± 0.6	(ns)	51.9 ± 0.3	52.1 ± 0.3	(ns)
	Trial 2	OTC	6:00-18:00	418.5 ± 1.5	785.4 ± 36.7	(***)	26.6 ± 0.6	25.8 ± 0.6	(ns)	47.1 ± 3.0	48.6 ± 3.0	(ns)
	Trial 3	OTC	6:00-18:00	418.4 ± 1.8	1047.6 ± 58.5	(***)	15.4 ± 0.6	15.6 ± 0.6	(ns)	61.7 ± 1.5	61.6 ± 1.5	(ns)
	Trial 4	OTC	6:00-18:00	417.1 ± 1.8	921.5 ± 28.7	(***)	25.1 ± 0.6	24.7 ± 0.6	(ns)	49.6 ± 2.5	52.9 ± 2.5	(ns)
Night time	Trial 1		19:00-5:00	475.2 ± 4.6	764.3 ± 2.7	(***)	17.8 ± 0.1	17.6 ± 0.1	(*)	58.5 ± 0.2	59.2 ± 0.2	(ns)
	Trial 2		18:00-6:00	441.8 ± 3.0	451.4 ± 4.5	(ns)	17.9 ± 0.5	17.6 ± 0.5	(ns)	75.5 ± 1.9	76.7 ± 1.8	(ns)
	Trial 3		18:00-6:00	433.6 ± 2.8	434.1 ± 2.9	(ns)	9.6 ± 0.5	9.6 ± 0.5	(ns)	84.9 ± 1.0	85.1 ± 0.9	(ns)
	Trial 4		18:00-6:00	442.4 ± 4.0	450.1 ± 4.2	(ns)	17.3 ± 0.4	17.2 ± 0.4	(ns)	75.0 ± 1.6	79.2 ± 1.6	(ns)

Data are expressed as mean ± standard error (S.E.).

Values in brackets are the results of *t*-test between a[CO_2] and e[CO_2] in each trial.

ns, no significant difference; *, *** significant difference at $P = 0.05$ and 0.001, respectively.

3.3 Results

Actual conditions in the chambers

In Table 3.1, actual conditions in the chambers were summarized. For all trials, mean CO_2 concentrations during the growth period at day time under e[CO_2] were approximately twice that under a[CO_2]. CO_2 was supplied at night only in GC, thus CO_2 concentrations were higher under e[CO_2] in Trial 1 than in other trials. Average temperature during the growth periods was similar in Trials 1, 2 and 4, approximately at 25 °C and 17 °C during the day and night, respectively. However, temperature was lower in Trial 3 comparing with the other three trials, approximately at 15 °C and 10 °C during the day and night, respectively. That is because Trial 2 was conducted in autumn season. Relative humidity was a little variation among these trials. For temperature and relative humidity, there was no significant difference between a[CO_2] and e[CO_2] in each independent trial, indicating possible variance of growth response in potato plants between a[CO_2] and e[CO_2] is caused by CO_2 concentration rather than other factors within each trial.

Plant appearance, total plant biomass and tuber biomass in Trial 1

On the 20 DAT, leaf color was a little dark under e[CO_2] than a[CO_2] in Control and LN, but not obviously different in LP (Figure 3.1A). Accelerated senescence by e[CO_2] was observed in all treatments, as shown on 75, 30, and 75 DAT in Control, LN, and LP, respectively. All plants were harvested until plant became entire senescence as shown in Figure 3.1A. Total plant biomass was increased by 1.4-fold under e[CO_2] only in Control (P = 0.003), but not significantly changed in LN (P = 0.051) and LP (P = 0.807) (Figure 3.1B and C). Similar with total biomass, tuber biomass was increased by 1.6-fold under e[CO_2] in Control (P = 0.007), but not in LN (P = 0.096) and LP (P = 0.913) (Figure 3.1D and E).

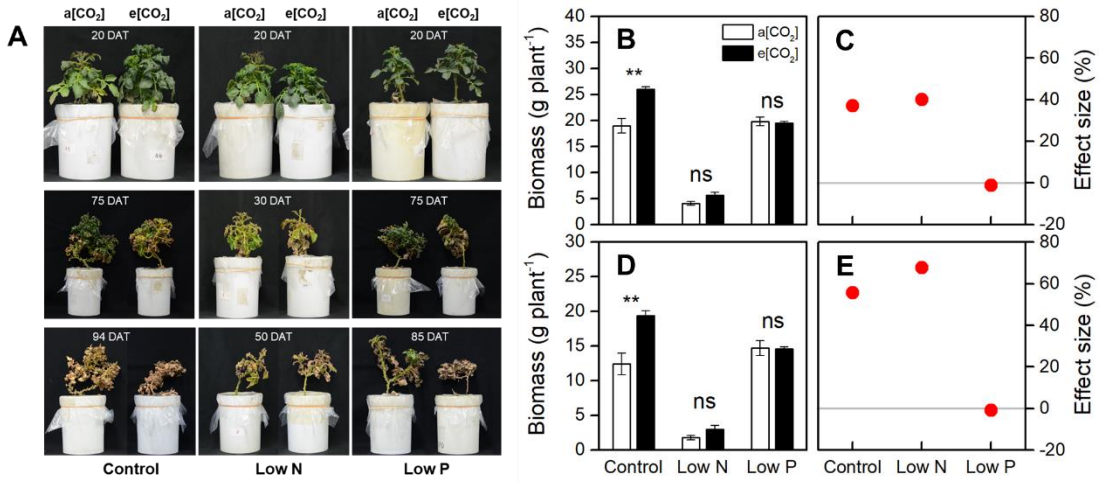

Figure 3.1 (A) Appearance of potato plants grown under a[CO_2] (387.9 ± 3.8 ppm) and e[CO_2] (781.9 ± 2.5 ppm) with sufficient nutrients (Control, 0.8 g N kg^{-1} soil and 1.5 g P kg^{-1} soil), Low N (LN, 0.1 g N kg^{-1} soil and 1.5 g P kg^{-1} soil), and Low P (LP, 0.8 g N kg^{-1} soil and 0.1 g P kg^{-1} soil) at different growth stages. **(B)** Biomass and **(C)** effect size of total plant at the harvest (94, 50, and 85 days after transplanting for Control, LN, and LP, respectively). **(D)** Biomass and **(E)** effect size of tuber at the harvest. Data in each plot are means ± S.E. (n = 4 biological replicates for each treatment).

Plant appearance, total plant biomass and tuber biomass in Trial 2

Plant appearance on the harvest day was shown in Figure 3.2A. According to the appearance, plant size in HP was larger than in LP. Plant size in LN was larger than in HN. That was because plants under HN likely suffered from salinity at early growth stage. The effect of K supply and CO_2 enrichment were not unclear from the appearance. Consistent with the plant appearance, total plant biomass and tuber biomass were significantly higher in HP than LP, but lower in HN than LN (Figure 3.2B and D). K supply did not change total plant biomass and tuber biomass, which may be due to excessive K supply.

Similar change of effect size of e[CO_2] was observed in total plant biomass and tuber biomass (Figure 3.2C and E). Effect size of e[CO_2] on total plant biomass ranged from -22% to 36% under various nutrient status (Figure 3.2C). There was a significant interaction between CO_2 and P supply on both total plant biomass ($P < 0.001$) and tuber biomass ($P = 0.011$) (Table S1), thus effect size on total plant biomass showed negative

value ranging from -22% to -5% under LP, however positive value ranging from 11% to 36% under HP (Figure 3.2C). Biomass in total plant and tuber was decreased by e[CO_2] in LP, however, increased in HP, indicating CO_2-fertilization effect was remarkably inhibited by P deficiency.

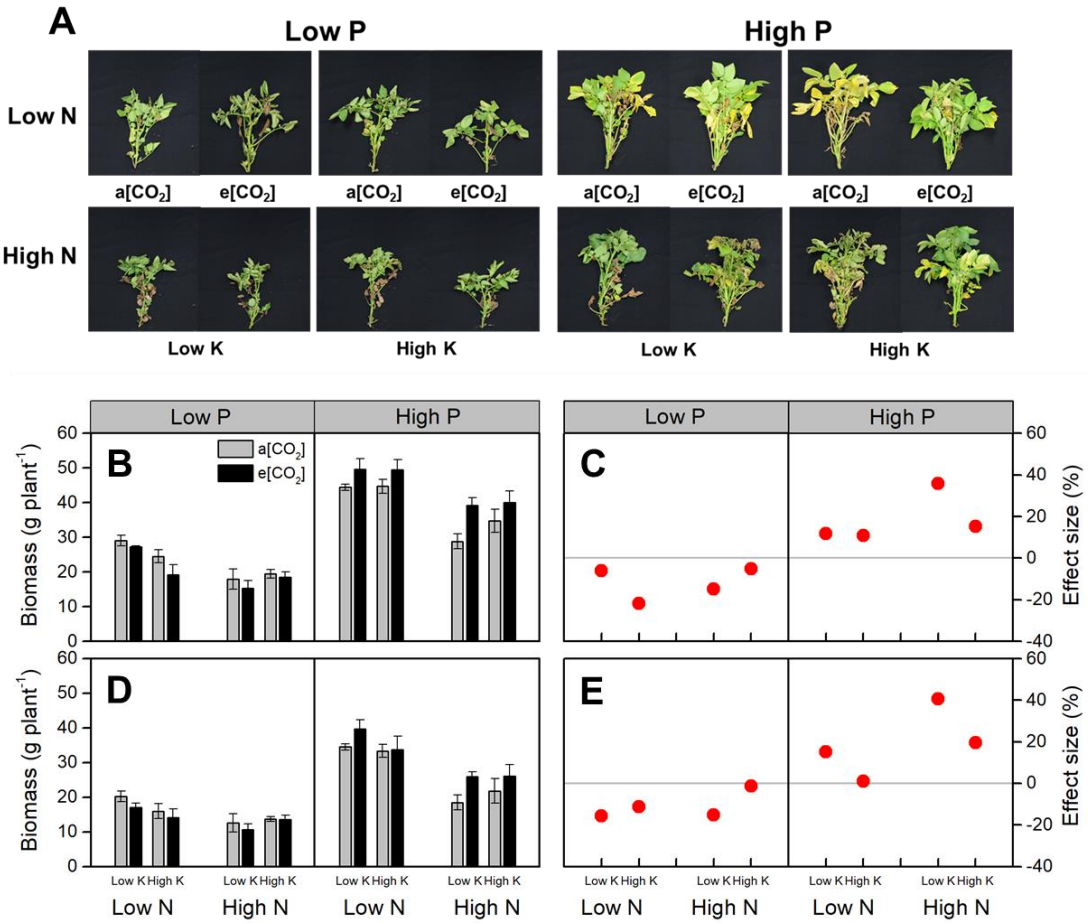

Figure 3.2 (A) Appearance of potato plants grown under a[CO_2] (418.5 ± 1.5 ppm) and e[CO_2] (785.4 ± 36.7 ppm) with combined nutrients supply (Low N: LN, 0.3 g N kg^{-1} soil; High N: HN, 3 g N kg^{-1} soil; Low P: LP, 0.3 g P kg^{-1} soil; High P: HP, 3 g P kg^{-1} soil; Low K: LK, 0.3 g K_2O kg^{-1} soil; High K: HK, 3 g K_2O kg^{-1}) at harvest day (58 days after transplanting). **(B)** Biomass and **(C)** effect size of total plant at the harvest. **(D)** Biomass and **(E)** effect size of tuber at the harvest. Data in each plot are means ± S.E. (n = 5 biological replicates for each treatment).

Plant appearance, total plant biomass and tuber biomass in Trial 3

Plants were sampled at three times, 26, 48, and 71 DAT, respectively. Plant appearance at sampling was shown in Figure 3.3A. According to the plant appearance, plant growth was inhibited under LP. The change of total plant biomass and tuber biomass was similar (Figure 3.3B and D). Effect size of e[CO_2] on both total plant and tuber biomass

was larger under HP than that under LP at each harvest (Figure 3.3C and E). Total plant and tuber biomass was increased by e[CO_2] under HP at all three stages; biomass was increased by e[CO_2] at 26 and 71 DAT, but decreased by e[CO_2] at 48 DAT under LP (Figure 3.3C and E). At 48 DAT, effect size was smaller than that at the other two stages, especially under LP. That may be related to both plant development and environmental changes, such as temperature (Figure S3.4).

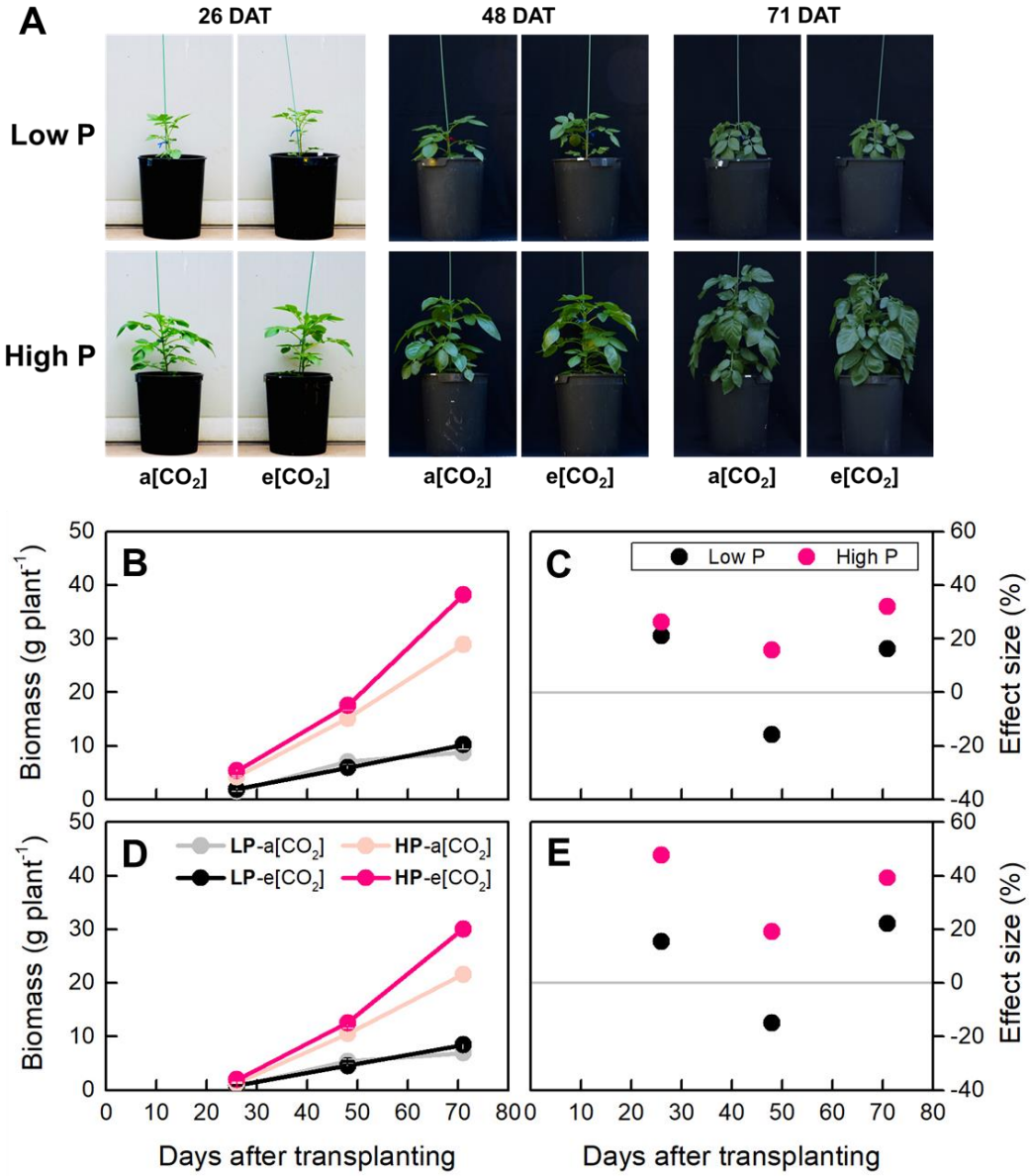

Figure 3.3 (A) Plant appearance on the harvest days (26, 48, and 71 days after transplanting (DAT)). **(B)** Total plant biomass, **(C)** effect size of e[CO_2] in total plant biomass, **(D)** tuber biomass, and **(E)** effect size of e[CO_2] in tuber biomass of potato plants grown under a[CO_2] and e[CO_2] with Low P (LP, 0.3 g P kg^{-1} soil) and High P (HP, 3 g P kg^{-1} soil) at different sampling times (26, 48, and 71 DAT). Data in each plot are means ± S.E. (n = 4 biological replicates for each treatment).

Leaf SPAD in Trial 3

SPAD value was reported to be significant consistent with chlorophyll concentration (Yamamoto et al., 2002). To estimate chlorophyll in leaves at different positions, SPAD of the oldest leaf blade of each node were measured (Figure 3.4). SPAD was higher under e[CO_2] than a[CO_2] for the upper leaves under both P supply at 26 DAT, and then decreased to a similar level with a[CO_2] under HP or lower level than a[CO_2] under LP at 71 DAT.

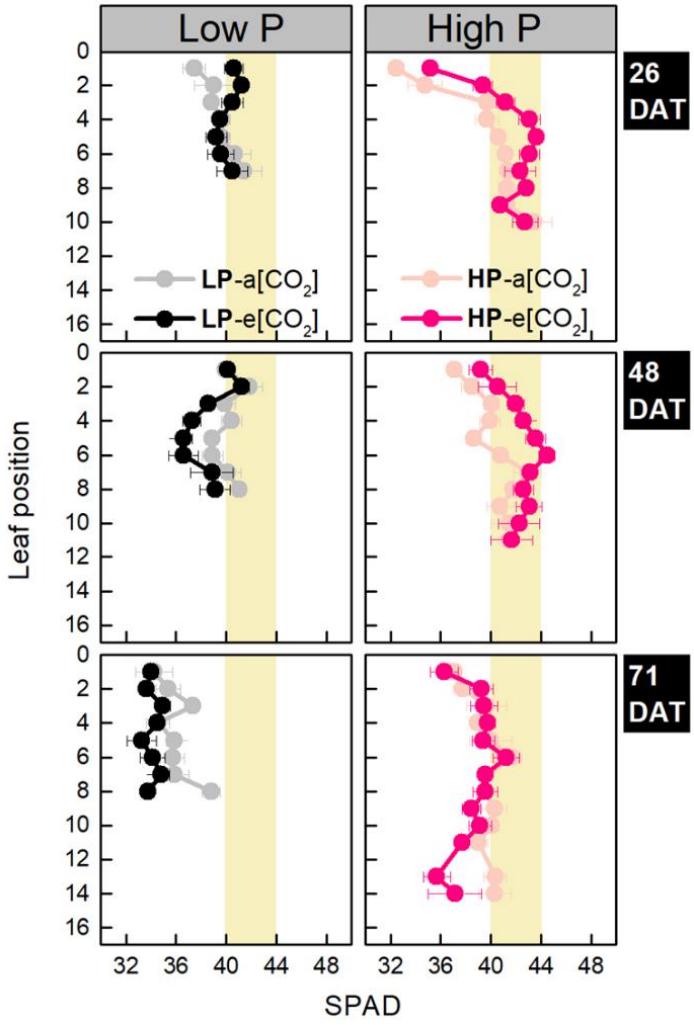

Figure 3.4 Leaf SPAD of potato plants grown under a[CO_2] and e[CO_2] with Low P (LP, 0.3 g P kg^{-1} soil) and High P (HP, 3 g P kg^{-1} soil) at different sampling times (26, 48, and 71 days after transplanting (DAT)). Data in each plot are means ± S.E. (n = 4 biological replicates for each treatment). Leaf position was counted from top to down. The oldest leaf blade of each compound leaf was measured.

Plant appearance, total plant biomass and tuber biomass in Trial 4

According to appearance of potato plant at the harvest day, there were no green leaves in plants under e[CO_2], however, some leaves of plants under a[CO_2] still kept green (Figure 3.5A). This indicates an accelerated senescence by e[CO_2].

Total plant biomass was decreased by e[CO_2] under both LP and HP according to ANOVA analysis (P = 0.014) (Figure 3.5B). Effect size of e[CO_2] on total plant biomass were -14% and -17% under LP and HP, respectively. Though tuber biomass was lower under e[CO_2] than a[CO_2], it was not significantly different (P = 0.091) (Figure 3.5C). Comparing with LP, biomass in total plant and tuber was higher under HP.

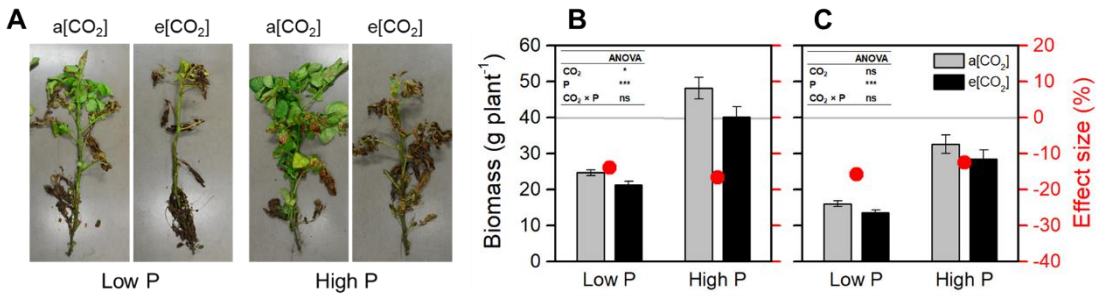

Figure 3.5 (A) Appearance of potato plants grown under a[CO_2] (417.1 ± 1.8 ppm) and e[CO_2] (921.5 ± 28.7 ppm) with Low P (LP, 0.3 g P kg^{-1} soil) and High P (HP, 3 g P kg^{-1} soil) at harvest day (55 days after transplanting). **(B)** Biomass and effect size of total plant and at the harvest. **(C)** Biomass and effect size of tuber at the harvest. Data in each plot are means ± S.E. (n = 8 biological replicates for each treatment). Statistical comparisons (two-way ANOVA) between CO_2 concentrations and P supply rates as well as their interaction (CO_2 × P) are presented.

Leaf nutrition status at harvest in Trial 4

Leaf [N] was not affected by e[CO_2] under both P supply rates (P = 0.950), however, it was lower in plants under HP than LP (Figure 3.6A), which could be attributed to dilution effect due to the great increase in biomass under HP.

Leaf [P] was similar between a[CO_2] and e[CO_2] under both P supply rates (P = 0.849) (Figure 3.6B). Leaf [P] under LP was approximately at 2 mg P g^{-1} DW, and 3.5

mg P g^{-1} DW under HP, indicating a success for controlling P deficient and sufficient conditions.

Leaf C/N was little affected by e[CO_2] under both P supply (Figure 3.6C). Leaf C/N was higher under HP than LP, which could result from lower leaf [N] under HP than LP (Figure 3.6A).

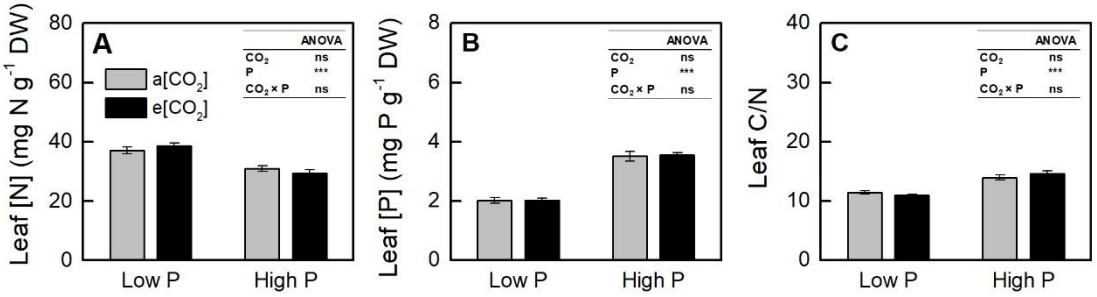

Figure 3.6 (A) Leaf nitrogen concentration ([N]), **(B)** leaf phosphorus concentration ([P]), and **(C)** leaf C/N of potato plants grown under a[CO_2] (417.1 ± 1.8 ppm) and e[CO_2] (921.5 ± 28.7 ppm) with Low P (LP, 0.3 g P kg^{-1} soil) and High P (HP, 3 g P kg^{-1} soil) at harvest day (55 days after transplanting). Data in each plot are means ± S.E. (n = 8 biological replicates for each treatment). Statistical comparisons (two-way ANOVA) between CO_2 concentrations and P supply rates as well as their interaction (CO_2 × P) are presented.

Starch, glucose, and sucrose in the youngest fully expanded leaf in Trial 4

Starch was increased by e[CO_2] only under HP (Figure 3.7A). Additionally, starch was also higher in plants under HP comparing with that under LP. Glucose was increased by e[CO_2] under both P supply rates, but not affected by P supply (Figure 3.7B). Sucrose was not affected by CO_2 and P supply (Figure 3.7C).

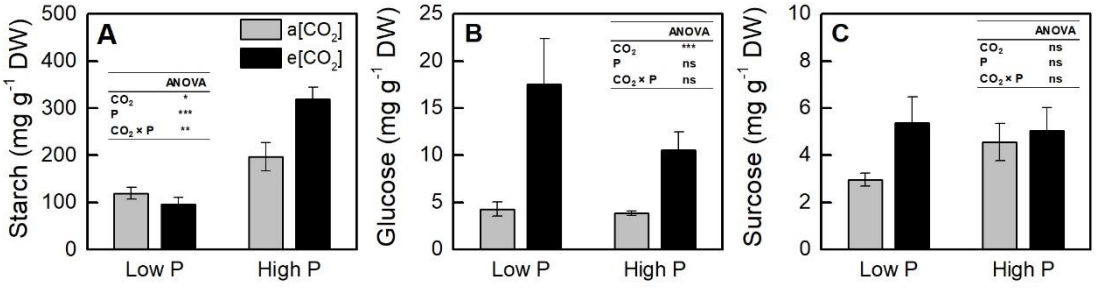

Figure 3.7 (A) Starch concentration, **(B)** glucose concentration, and **(C)** sucrose concentration in the youngest fully expanded leaf of potato plants grown under a[CO_2] (417.1 ± 1.8 ppm) and e[CO_2] (921.5 ± 28.7 ppm) with Low P (LP, 0.3 g P kg^{-1} soil) and High P (HP, 3 g P kg^{-1} soil) at 48 days after transplanting. Data in each plot are means ± S.E. (n = 8 biological replicates for each treatment). Statistical comparisons (two-way ANOVA) between CO_2 concentrations and P supply rates as well as their interaction (CO_2 × P) are presented.

3.4 Discussion

In this study, I focused on how nutrient supply affects potato response to e[CO_2] at different developmental stages and chambers. It seems inappropriate nutrient management have been implemented in Trial 2. Plant biomass was lower under HN than LN and apparent salinity stress was observed in plants with HN at early growth. As for K supply, biomass was little affected though leaf K concentration was higher under HK than LK (Figure S3.7C). Though apparent salinity stress with high K was not observed, excessive K addition at one time may hurt plant growth. The adverse effects on plant growth from inappropriate nutrient supply was settled by basal application and topdressing of fertilizers in other trials (Trails 1, 3, and 4). Leaf nutrition status at each harvest was examined (Figures S3.6-3.8). Since effect size rather than the absolute growth was used to estimate CO_2 effects, the inappropriate nutrient management in Trial 2 is supposed to little affect the conclusions in this study.

1.4- and 1.6-fold increases in total plant and tuber biomass during long-term CO_2 enrichment covering the entire life cycle in growth chambers

Total plant growth and tuber biomass were enhanced by 1.4- and 1.6-fold under e[CO_2] in potato plants with sufficient nutrients in GC (Figure 3.1). Comparing with 1.5-fold increase by e[CO_2] in total plant for the maximum plant growth in Chapter 2.1, increment of total plant by e[CO_2] was a little lower in this study (1.4-fold). That may be related to down-regulation of photosynthesis after long-term exposure to e[CO_2]. CO_2 effects have been already reported smaller at the final harvest than that at intermediate harvest in potato (Craigon et al., 2002). A review research on soybean suggested that effect size of e[CO_2] was quite different depending on growth stages (Ainsworth et al., 2002).

In Trial 1, leaf color became yellow more seriously under e[CO_2] than a[CO_2] at the same growth period under each nutrient treatment (Figure 3.1A). Miglietta et al (1998) have already reported accelerated leaf senescence under e[CO_2] in a FACE

experiment. Bindi et al. (2006) in an experiment conducted at the same site, also found that canopy senescence was accelerated under e[CO_2] although crop phenology was not affected. It has been already reported that biomass accumulation can be stimulated by e[CO_2], however, accelerated senescence will limit it at the end of the growing season (Finnan et al., 2005). Despite of the accelerated senescence by e[CO_2], plant growth was enhanced but only when nutrients were sufficient in Trial 1 (Figure 3.1). Total plant biomass in LP was not increased by e[CO_2], and at the margin test value in LN (P = 0.051). As N and P are important macronutrients in plant, the effects of e[CO_2] were always dependent on soil N (Kitao et al., 2005) and P availability (Campbell and Sage, 2006).

Lower effect size of e[CO_2] on potato growth in open-top chambers than in growth chambers, especially under phosphorus deficiency

To further confirm CO_2 effects on potato growth at a relatively large scale and natural conditions, three experiments were conducted in OTC. The effect size of e[CO_2] on total plant biomass at entire maturity was minus in OTC but plus in GC (Figures 3.1 and 3.5). Additionally, at intermediate harvest in OTC in Trial 2, e[CO_2] decreased plant growth under LP, but increased growth under HP (Figure 3.2). The overall results in this study indicate CO_2 effects on potato growth was quite different at different developmental stages and in various CO_2 enrichment facilities; effect size of e[CO_2] was lower in OTC than in GC, especially under LP.

Accelerated senescence under e[CO_2] was observed in both GC (Figure 3.1A) and OTC (Figure 3.5A), however, plant growth was decreased by e[CO_2] only in OTC (Figure 3.5). It is still uncertain what caused the different responses to e[CO_2] between GC and OTC, since there were several differences between them, such as more fluctuation in CO_2 concentration in OTC than GC, short day time for CO_2 fumigation in OTC (6:00 – 18:00) than GC (5:00 – 19:00), and no CO_2 enrichment at night in OTC but have in GC (Table 3.1, Figures S3.2 and S3.5). Additionally, irradiance was

speculated varied between the two CO_2 enrichment facilities, which was reported to affect CO_2 effects on potato growth (Wheeler et al., 1991). Despite many uncertain factors, an important reason resulting in lower effect size in OTC than in GC may be the altered lifespans. It took approximate three months for plants in GC to grow entire senescence under nutrient sufficiency (Figure 3.1), however, only two months in OTC (Figure 3.5).

It is worth pointing out that accelerated senescence under e[CO_2] was only observed in Trial 4, though Trials 2, 3, and 4 were conducted in OTC. Trials 2 and 4 were carried out in the warm seasons and had similar growth period (Table 3.1), but the exposure period to e[CO_2] was longer in Trial 4 (55 days, Figure S3.5) than in Trial 2 (43 days, Figure S3.3) because CO_2 fumigation started 15 DAT in Trial 2 due to equipment malfunction. That may explain the early senescence under e[CO_2] in Trial 4 but not in Trial 2. As for Trial 3, it was carried out in the cold season with low temperature (Table 3.1, Figure S3.4). Therefore, no any symptom of senescence was observed in this experiment even plants grew for 71 days (Figure 3.3A) due to extended life cycle. That is because plant grow slowly at low temperature (Nagai and Makino, 2009). Besides of temperature, short daytime and low irradiance in autumn season may also impact plant development (Adams and Langton, 2005; Warrington et al., 1976).

Accelerated senescence under e[CO_2] involving in sugar-regulated senescence rather than imbalanced C/N ratio

Accelerated senescence by e[CO_2] was observed in Trial 4 under both LP and HP (Figure 3.5A), indicating negative CO_2 effects on plant biomass accumulation may be related to earlier senescence under e[CO_2]. Accelerated senescence under e[CO_2] have been already reported in several species besides potato (Bindi et al., 2006), such as sunflower (de la Mata et al. 2012), and Arabidopsis (Aoyama et al., 2014).

The e[CO_2] usually causes accumulation of carbohydrates, especially starch, which decreases N concentration, thereby leading to an imbalanced C/N ratio in mature

leaves (Ainsworth and Long, 2005; Vicente et al. 2016). Some studies suggested that imbalanced C/N ratio may be the main reason to cause senescence in plants (Agüera et al., 2010; Agüera and de la Haba, 2018; Chen et al., 2015), which was supported by Aoyama et al. (2014), who observed accelerated senescence under e[CO_2] with limited N. However, that study was conducted during a shot-term growth period, thus the plants with sufficient N preserved leaf greenness may be due to extended life cycle. That is to say, accelerated senescence under e[CO_2] may also be observed in plants with sufficient N when extending growth period like in this study (Figures 3.1 and 3.5). Despite of the increased starch under e[CO_2] in Trial 4 (Figure 3.7), leaf C/N was little affected by e[CO_2] (Figure 3.6). Additionally, leaf C/N was also little affected by e[CO_2] in Trial 1 (Figure S3.6C), in which senescence was also accelerated by e[CO_2] (Figure 3.1A). Therefore, e[CO_2]-induced senescence is hardly to be explained by imbalanced C/N ratio in this study.

Sugar signaling pathway plays a major role in the regulation of senescence (Wingler et al., 2006; Wingler and Roitsch 2008). In Trial 4, leaf sucrose was little affected by e[CO_2] (Figure 3.7). As for glucose, e[CO_2] remarkably increased it under both LP and HP. Combining with the apparent senescence under e[CO_2], it is likely the accelerated senescence in Trial 4 was related to glucose accumulation in leaves. However, further research is needed to confirm this.

The e[CO_2]-induced senescence cannot be avoided but can be delayed by nutrient supply due to extended life cycle

Accelerated senescence under e[CO_2] in potato plants was clearly observed in this study (Figures 3.1 and 3.5), which have also been reported in some previous studies (Bindi et al., 2006; de la Mata et al., 2012). The effects of e[CO_2]-induced accelerated senescence on plant biomass accumulation varied among the experiments in this study; growth was promoted by e[CO_2] even senescence was accelerated under sufficient nutrient conditions in GC (Figure 3.1), however, biomass was decreased by e[CO_2] in OTC due

to earlier senescence even under sufficient nutrient conditions (Figure 3.5). In Trial 3, plants were harvested at different developmental stages before any symptom of senescence; the change of leaf SPAD indicates positive effects of e[CO_2] may disappear as plants grow even under HP (Figure 3.4). That indicates that e[CO_2]-induced senescence cannot be avoided but may be delayed by nutrient supply.

3.5 Conclusions

This study aimed to investigate long-term CO_2 effects covering the entire growth period and examine effect size of e[CO_2] in different CO_2 enrichment facilities and at different developmental stages under various nutrient conditions. After carrying out experiments, I found that CO_2-fertilization effect was inhibited in a certain degree at the late development stage comparing with the results in Chapter 2. Furthermore, the decreased CO_2 effect at late stage is likely related to accelerated senescence under e[CO_2], especially in OTC. Though the reasons for varied degree of accelerated senescence under GC and OTC is still unclear, e[CO_2]-induced senescence is likely related to sugar accumulation in leaves rather than imbalance C/N ratio. In this study, nutrient supply could not prevent accelerated senescence by e[CO_2] from the whole growth cycle (Figures 3.1 and 3.5), but it may delay it by extending growth period (Figures 3.1 and 3.4).

Table S1

Table S1 Output of four-way ANOVA for the measurements of potato plants in Trial 2.

	Total plant biomass	Tuber biomass	Leaf [N]	Leaf [P]	Leaf [K]	Leaf C/N
CO_2	0.116	0.276	0.156	0.845	0.005	0.003
N	< 0.001	< 0.001	< 0.001	< 0.001	0.908	< 0.001
P	< 0.001	< 0.001	< 0.001	< 0.001	0.051	< 0.001
K	0.916	0.459	0.017	0.019	< 0.001	0.900
CO_2 × N	0.338	0.337	0.005	0.179	0.199	0.002
CO_2 × P	< 0.001	0.011	0.974	0.382	0.020	0.068
CO_2 × K	0.434	0.593	0.464	0.092	0.167	0.197
N × P	0.079	0.001	< 0.001	< 0.001	< 0.001	< 0.001
N × K	0.013	0.023	< 0.001	0.078	0.003	0.247
P × K	0.120	0.936	0.273	0.924	< 0.001	0.840
CO_2 × N × P	0.822	0.724	0.062	0.833	0.966	0.020
CO_2 × N × K	0.946	0.830	0.169	0.822	0.066	0.136
CO_2 × P × K	0.710	0.231	0.247	0.732	0.830	0.541
N × P × K	0.260	0.957	0.006	0.802	0.835	0.453
CO_2 × N × P × K	0.291	0.887	0.248	0.527	0.974	0.959

Table S2

Table S2 Output of ANOVA for the measurements of potato plants in Trial 3.

	Total biomass	Tuber biomass	Leaf [N]	Leaf [P]	Leaf C/N	Starch	Glucose	Sucrose
Stage	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.758
CO_2	< 0.001	< 0.001	< 0.001	0.052	< 0.001	0.013	0.006	0.054
P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.001	0.061	0.012
Stage × CO_2	< 0.001	< 0.001	< 0.001	0.005	0.076	0.046	0.208	0.200
Stage × P	< 0.001	< 0.001	0.362	< 0.001	0.025	0.109	0.992	0.248
CO_2 × P	< 0.001	< 0.001	0.113	0.431	0.068	0.202	0.022	0.131
Stage × CO_2 × P	0.016	0.004	0.082	0.111	0.167	0.050	0.056	0.278
Stage 1 (26 DAT)								
CO_2	0.004	0.006	< 0.001	0.022	0.003	0.102	0.014	0.038
P	< 0.001	< 0.001	< 0.001	< 0.001	0.005	0.786	< 0.001	0.690
CO_2 × P	0.086	0.043	0.532	0.473	0.907	0.828	0.229	0.131
Stage 2 (48 DAT)								
CO_2	0.511	0.357	< 0.001	0.078	< 0.001	0.023	0.001	0.037
P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.019	0.189	0.103
CO_2 × P	0.084	0.044	0.015	0.121	0.001	0.040	0.509	0.059
Stage 3 (71 DAT)								
CO_2	< 0.001	< 0.001	0.619	0.045	0.315	0.491	0.357	0.802
P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.003	0.474	0.067
CO_2 × P	0.003	0.002	0.439	0.116	0.523	0.288	0.049	0.782

Figure S3.1

Figure S3.1 The picture of the open-top chambers.

Figure S3.2

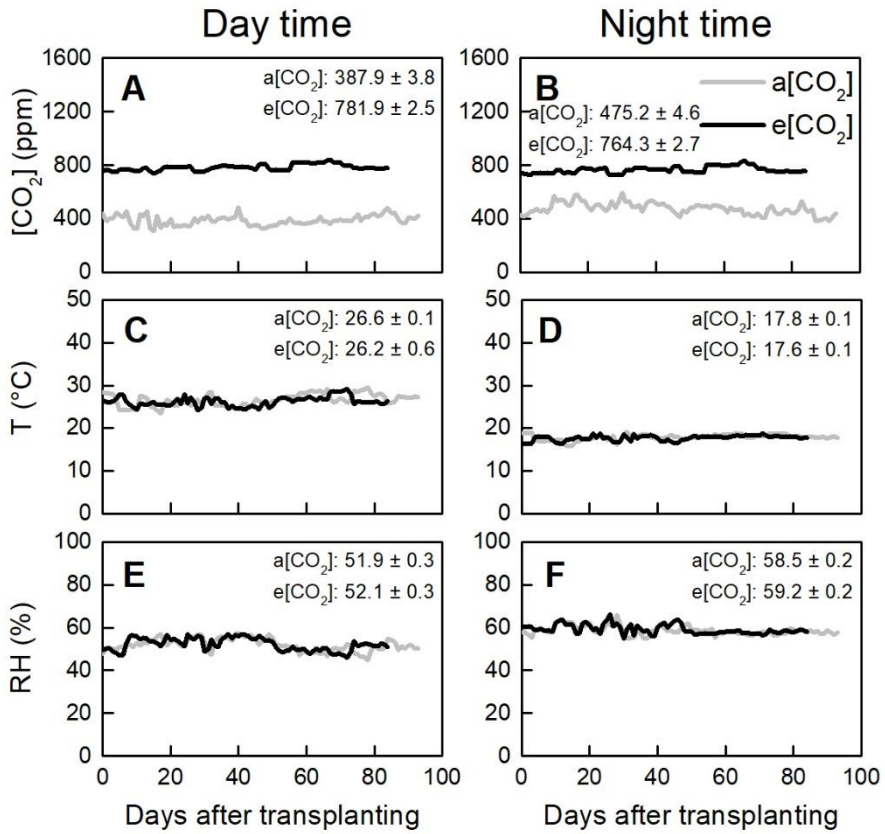

Figure S3.2 Actual conditions in growth chambers during growth period in Trial 1. Actual CO_2 concentrations at day time **(A)** and night time **(B)**. Actual temperature (T) at day time **(C)** and night time **(D)**. Actual relative humidity (RH) at day time **(E)** and night time **(F)**. The means and standard error during the growth period were presented.

Figure S3.3

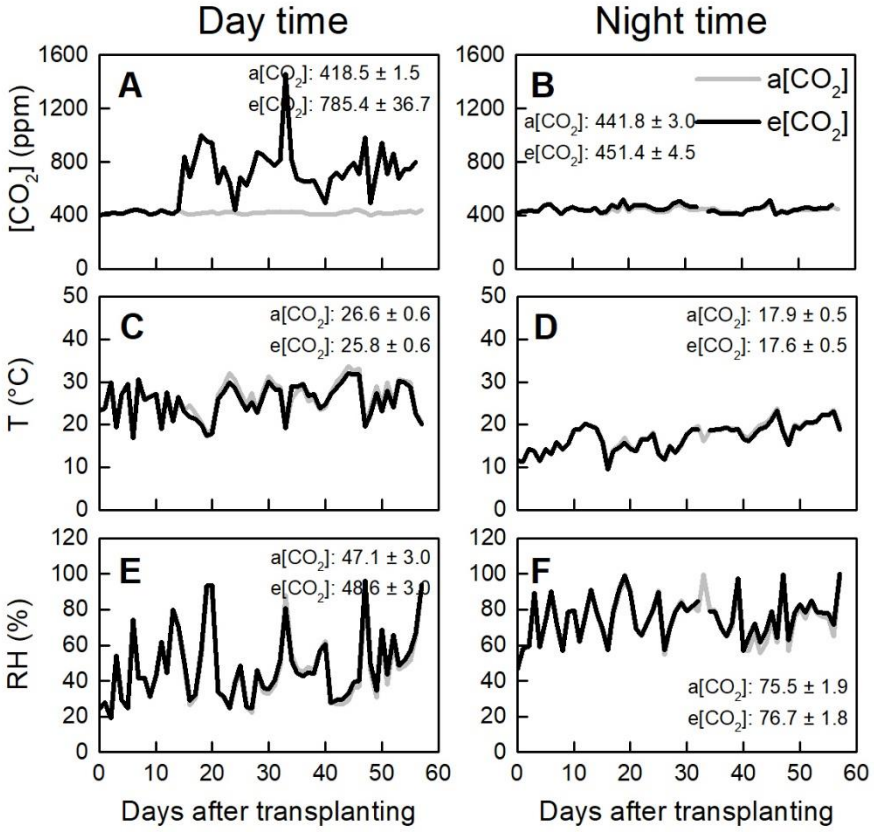

Figure S3.3 Actual conditions in open-top chambers during growth period in Trial 2. Actual CO_2 concentrations at day time **(A)** and night time **(B)**. Actual temperature (T) at day time **(C)** and night time **(D)**. Actual relative humidity (RH) at day time **(E)** and night time **(F)**. The means and standard error during the growth period were presented.

Figure S3.4

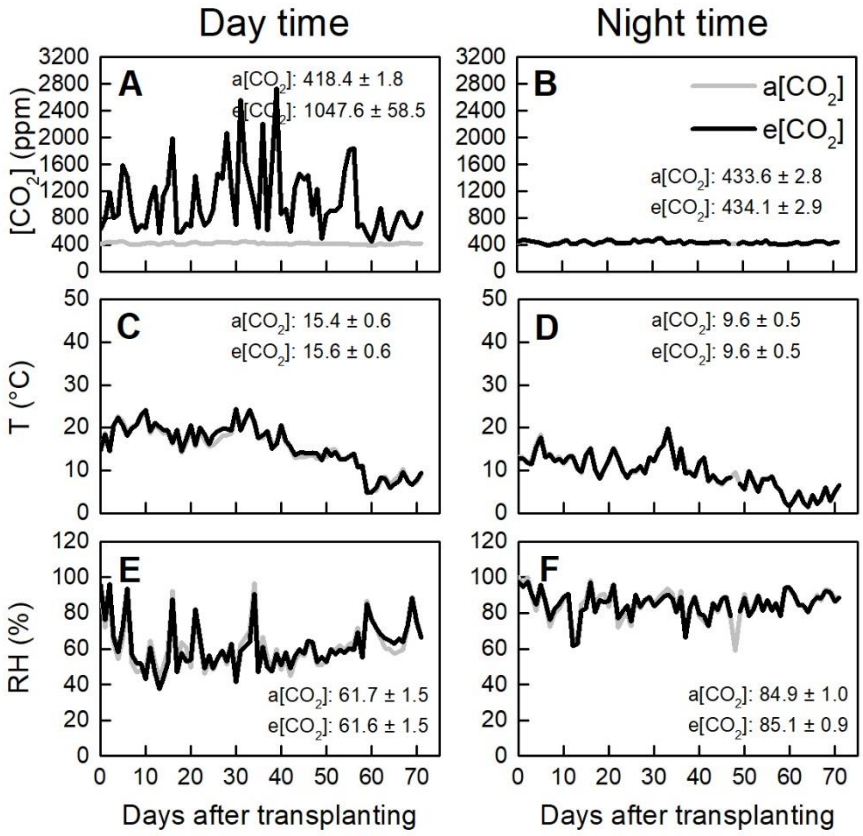

Figure S3.4 Actual conditions in open-top chambers during growth period in Trial 3. Actual CO_2 concentrations at day time **(A)** and night time **(B)**. Actual temperature (T) at day time **(C)** and night time **(D)**. Actual relative humidity (RH) at day time **(E)** and night time **(F)**. The means and standard error during the growth period were presented. Missing data was unavailable due to malfunction of the recorder. CO_2 concentration fluctuated greatly under e[CO_2] due to malfunction of gas controller.

Figure S3.5

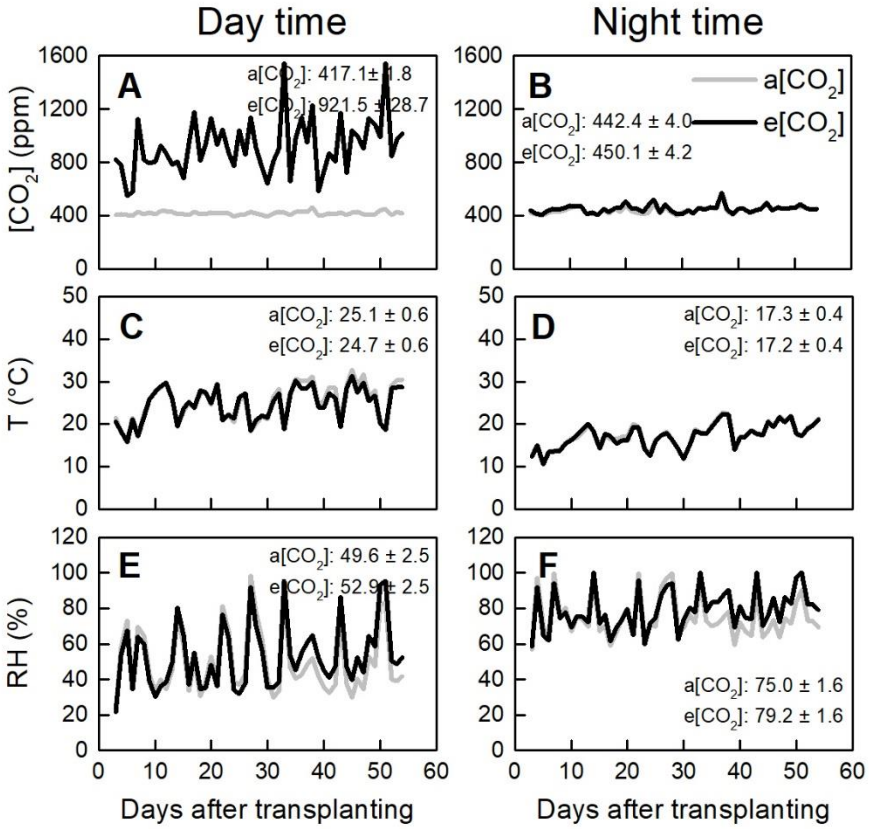

Figure S3.5 Actual conditions in open-top chambers during growth period in Trial 4. Actual CO_2 concentrations at day time **(A)** and night time **(B)**. Actual temperature (T) at day time **(C)** and night time **(D)**. Actual relative humidity (RH) at day time **(E)** and night time **(F)**. The means and standard error during the growth period were presented.

Figure S3.6

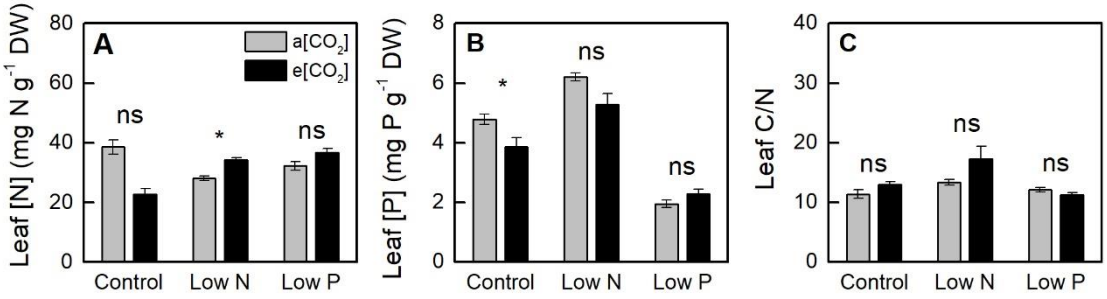

Figure S3.6 (A) Leaf nitrogen concentration ([N]), **(B)** leaf phosphorus concentration ([P]), and **(C)** leaf C/N of potato plants grown under a[CO_2] (387.9 ± 3.8 ppm) and e[CO_2] (781.9 ± 2.5 ppm) with sufficient nutrients (Control, 0.8 g N kg^{-1} soil and 1.5 g P kg^{-1} soil), Low N (LN, 0.1 g N kg^{-1} soil and 1.5 g P kg^{-1} soil), and Low P (LP, 0.8 g N kg^{-1} soil and 0.1 g P kg^{-1} soil). Data in each plot are means ± S.E. (n = 4 biological replicates for each treatment).

Figure S3.7

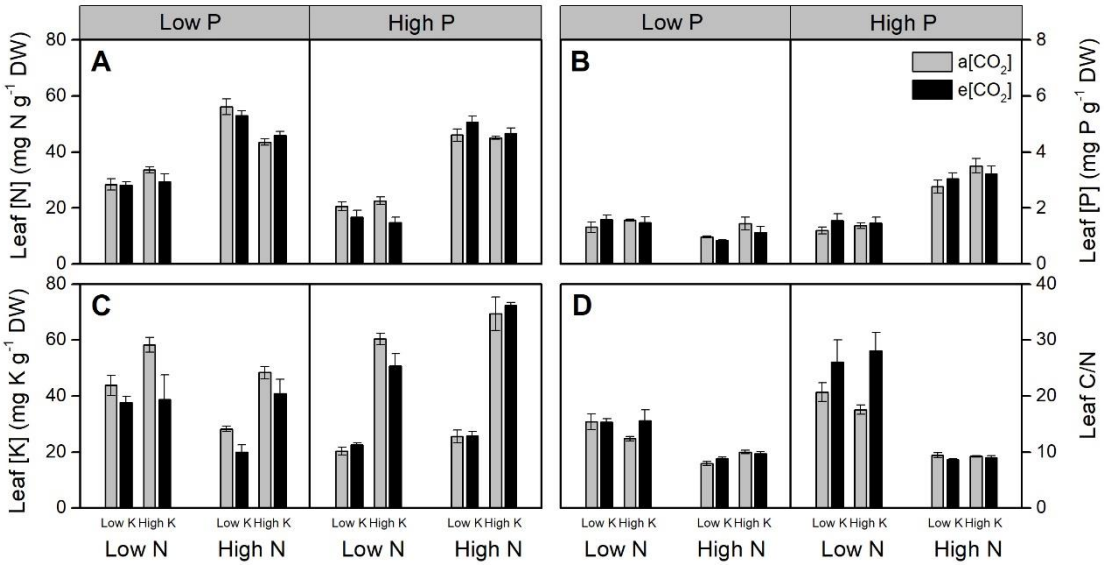

Figure S3.7 (A) Leaf nitrogen concentration ([N]), **(B)** leaf phosphorus concentration ([P]), **(C)** leaf potassium concentration ([K]), **(D)** leaf C/N of potato plants under a[CO_2] (418.5 ± 1.5 ppm) and e[CO_2] (785.4 ± 36.7 ppm) with combined nutrients supply (Low N: LN, 0.3 g N kg^{-1} soil; High N: HN, 3 g N kg^{-1} soil; Low P: LP, 0.3 g P kg^{-1} soil; High P: HP, 3 g P kg^{-1} soil; Low K: LK, 0.3 g K_2O kg^{-1} soil; High K: HK, 3 g K_2O kg^{-1}) at harvest. Data in each plot are means ± S.E. (n = 5 biological replicates for each treatment).

Figure S3.8

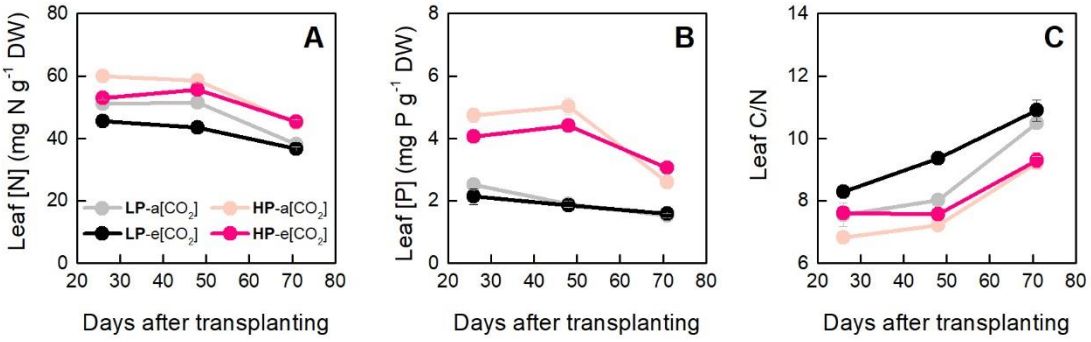

Figure S3.8 (A) Leaf nitrogen concentration ([N]), **(B)** leaf phosphorus concentration ([P]), and **(C)** leaf C/N of potato plants grown under a[CO_2] and e[CO_2] with Low P (LP, 0.3 g P kg^{-1} soil) and High P (HP, 3 g P kg^{-1} soil) at different sampling times (26, 48, and 71 days after transplanting). Data in each plot are means ± S.E. (n = 4 biological replicates for each treatment).

Chapter 4

Varietal difference of potato in response to elevated CO_2

4.1 Introduction

Potato, featuring with large sink capacity, is thought to be greatly profit from e[CO_2] (Marschner, 1995). In Chapter 3, effects of e[CO_2] on potato growth in a single variety (Irish Cobbler) were greatly affected by chamber types, which may be due to severe e[CO_2]-induced senescence in OTC than GC. Moreover, nutrient supply did not prevent e[CO_2]-induced senescence but delayed it due to extended lifespan. Early senescence may reduce crop yield; delay senescence is supposed to contribute to higher yields (Gregersen et al., 2013). Therefore, it is expected to profit more from e[CO_2] by delaying senescence in crops.

A simulation model of potato growth showed that yield could be stimulated by 20% for the late varieties and 30% for the early varieties under a doubling CO_2 (Schapendonk and Goudriaan, 1995). In contrast to the simulation study, the observed data on varietal difference in potato showed that CO_2 effects on yield was higher in the late variety than the early one (Schapendonk et al., 2000). The discrepancy between the simulation and the real experiment has been discussed as that more leaf area is expected under e[CO_2] in the simulation, however it is actually little affected by e[CO_2] (Schapendonk et al., 2000). They indicated that the late varieties are likely to have more potential to profit from e[CO_2], because late varieties have higher tuber production rates, which can provide a more durable sink for the enhanced carbohydrate production under e[CO_2].

Enhancement of crop growth by e[CO_2] varied within species, such as rice (Tsukaguchi et al., 2011; Zhang et al., 2015), French bean (Srinivasa Rao et al., 2015), wheat (Fernando et al., 2014) and potato (Schapendonk et al., 2000). In most studies, varietal differences to e[CO_2] have been thought to be related to sink capacity or sink production efficiency (Hasegawa et al., 2013; Yoshinaga et al., 2020). Little study associates CO_2 effects to the duration of green leaf or earliness of variety. Miller et al. (1997) suggested down-regulation of photosynthesis under e[CO_2] is the result of earlier shift in the timing of the normal photosynthetic stages of leaf ontogeny

associated with senescence. That indicates the importance of keeping greenness of leaves in crop production under e[CO_2].

Based on the results in Chapter 3 that nutrient supply did not prevent e[CO_2]-induced senescence but delayed it due to extended lifespan, I hypothesized that the late variety with longer lifespan can profit more from e[CO_2] comparing with the early one. In this study, six potato varieties belonging to distinct maturity groups were used to investigate the effects of variety earliness on CO_2 effects.

4.2 Materials and methods

Experimental design and growing conditions

A pot experiment was carried out in OTC located in Nagoya University in 2020 from the 17th, October to the 16th, December. The actual conditions in the chambers were monitored every 5 min (Figure 4.1). Average CO_2 concentration for the day time during the growth period were 414.5 and 750.8 ppm under a[CO_2] and e[CO_2], respectively. Average CO_2 concentration at night time were similar under both CO_2 conditions, approximately at 430 ppm. Both temperature and relative humidity were similar under the two CO_2 conditions as shown in Figure 4.1.

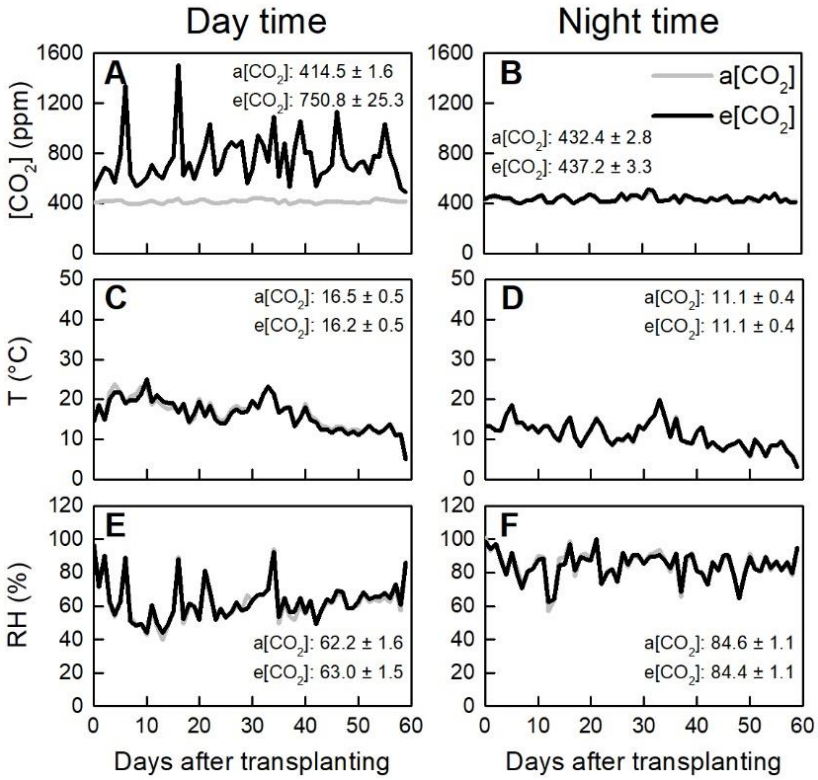

Figure 4.1 Actual conditions in growth chambers during growth period. Actual CO_2 concentrations at day time **(A)** and night time **(B)**. Actual temperature (T) at day time **(C)** and night time **(D)**. Actual relative humidity (RH) at day time **(E)** and night time **(F)**. The means and standard error during the growth period were presented.

Six potato varieties belonging to distinct maturing groups (https://www.jrt.gr.jp/var/var.html), very early (cv. Alowa, and Inkanomezame), early

(cv. Irish Cobbler, and Touya), medium-late (cv. Dejima), and late (cv. Red Moon), were used in this study. Naturally sprouted potato tubers were transplanted into 7.4-L pots (diameter, 22 cm; depth, 25 cm; one plant per pot) filled with 3.2 kg of dry andosol. Before transplanting, N (0.5 g N kg^{-1} dry soil), P (3.0 g P kg^{-1} dry soil), and K (1.2 g K_2O kg^{-1} dry soil) were uniformly mixed with the soil in the form of potassium chloride (60% K_2O), urea (46.0% N), and calcium superphosphate (17.5% P_2O_5), respectively. As topdressing, 0.25 g N kg^{-1} dry soil and 0.6 g K_2O kg^{-1} dry soil dissolved in tap water was added to each pot at 12 DAT. At 31 DAT, 0.25 g N kg^{-1} dry soil was applied again. The experiment was organized following a factorial design (two CO_2 concentrations × six varieties) with four biological replicates.

Harvest and sampling

All plants were harvested on the 60 DAT. Number of nodes, plant height, and leaf area were measured after harvesting. Leaves, stems, roots, and tubers were separated and then dried to constant weight in an oven at 80°C. Separated organs were ground to powder for N, P, or NSC quantification after dry weight determination.

Relative growth rate, net assimilation rate, and leaf area ratio

Relative growth rate (RGR), net assimilation rate (NAR), and leaf area ratio (LAR) in the growth period from transplanting (t1, 0 DAT) to harvest (t2, 60 DAT) were calculated following Shinjo et al. (2020),

$$\mathrm{RGR} = \frac{\ln \mathrm{W(t2)} - \ln \mathrm{W(t1)}}{\mathrm{t2} - \mathrm{t1}}$$

$$\mathrm{NAR} = \frac{\mathrm{W(t2)} - \mathrm{W(t1)}}{\mathrm{t2} - \mathrm{t1}} \times \frac{\ln \mathrm{LA(t2)} - \ln \mathrm{LA(t1)}}{\mathrm{LA(t2)} - \mathrm{LA(t1)}}$$

$$\mathrm{LAR} = \frac{\mathrm{LA(t2)} - \mathrm{LA(t1)}}{\ln \mathrm{LA(t2)} - \ln \mathrm{LA(t1)}} \times \frac{\ln \mathrm{W(t2)} - \ln \mathrm{W(t1)}}{\mathrm{W(t2)} - \mathrm{W(t1)}}$$

Where W(t) means plant biomass at time t; LA(t) means leaf area at time t.

Statistical analysis

The experiment was organized following a factorial design with two CO_2 concentrations and six varieties with four biological replicates (except for two replicates for Dejima under e[CO_2], three replicates for Alowa and Dejima under a[CO_2], because some plants in these treatments died after transplanting). Data are expressed as mean ± standard error (S.E.). Data were analyzed in SPSS 16.0 (SPSS Inc., Chicago, IL, USA) using two-way ANOVA at the 0.05 probability level. Effect size (%) = (e[CO_2] $_{biomass}$ – a[CO_2] $_{biomass}$) × 100%/a[CO_2] $_{biomass}$ (Ainsworth et al., 2002).

4.3 Results

Plant appearance and morphological characters

From the plant appearance at the harvest, plant height and size were clearly increased under e[CO_2] comparing with a[CO_2] in Touya, Dejima, and Red Moon (Figure 4.2A). Consistent with plant appearance, plant height and leaf area were increased under e[CO_2] and varied in different varieties (Figure 4.2C and D). Number of nodes slightly varied among the varieties examined, however, it was little affected by e[CO_2] (Figure 4.2B). There was no significant interaction between CO_2 and variety on number of node, plant height, and leaf area.

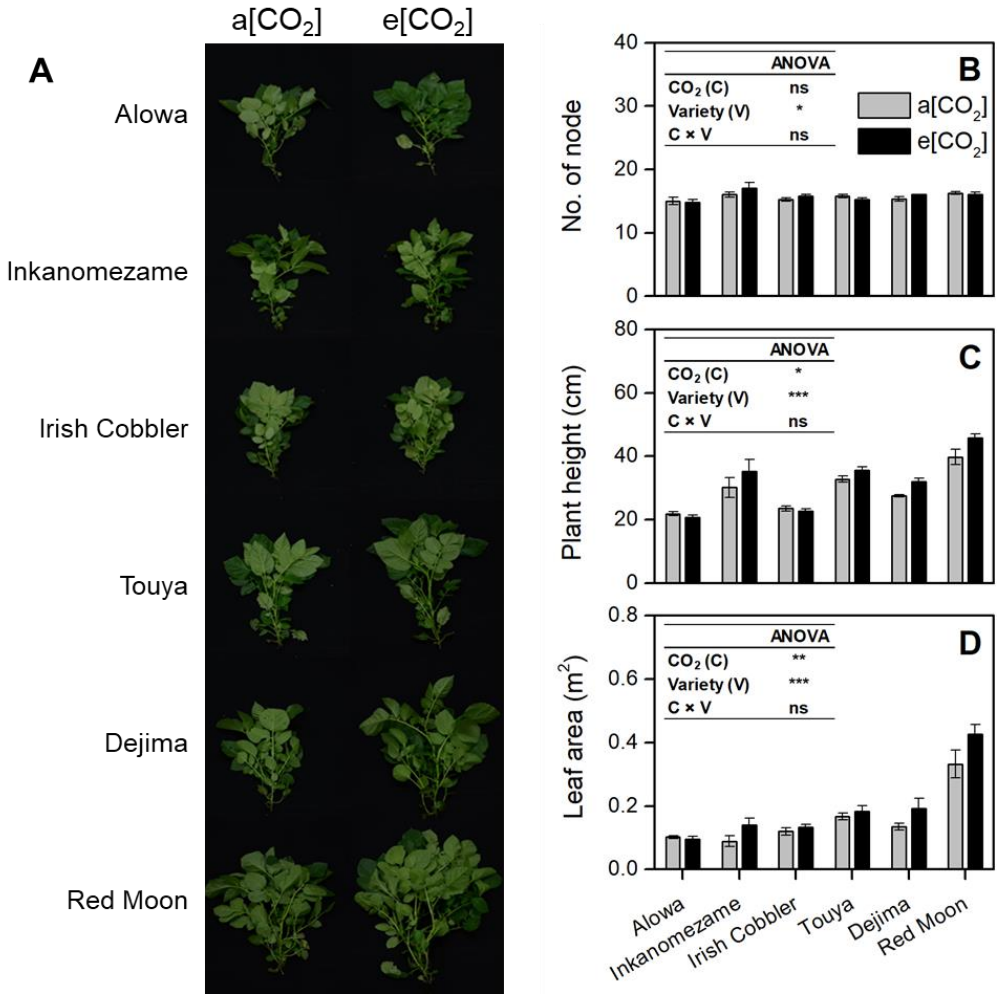

Figure 4.2 (A) Plant appearance at harvest on the 60 days after transplanting. **(B)** Number of node (No. of node), **(C)** plant height, and **(D)** leaf area of potato plants grown under a[CO_2] (414.5 ± 1.6 ppm) and e[CO_2] (750.8 ± 25.3 ppm). Statistical comparisons (two-way ANOVA) between CO_2 (C) and variety (V) as well as their interaction (C × V) are presented.

Plant biomass and effect size of e[CO_2]

Total plant and tuber biomass varied among varieties (Figure 4.3). The late maturity variety Red Moon had the largest biomass accumulation. Small biomass accumulation in medium-late variety Dejima is likely due to smaller plant size of Dejima at transplanting comparing with other varieties (Figure S4.1). Significant interaction between CO_2 and variety was observed in total plant biomass (P = 0.036). Effect size of e[CO_2] was largest in Red Moon, followed with Dejima and Inkanomezame. The variety Alowa had the smallest effect size.

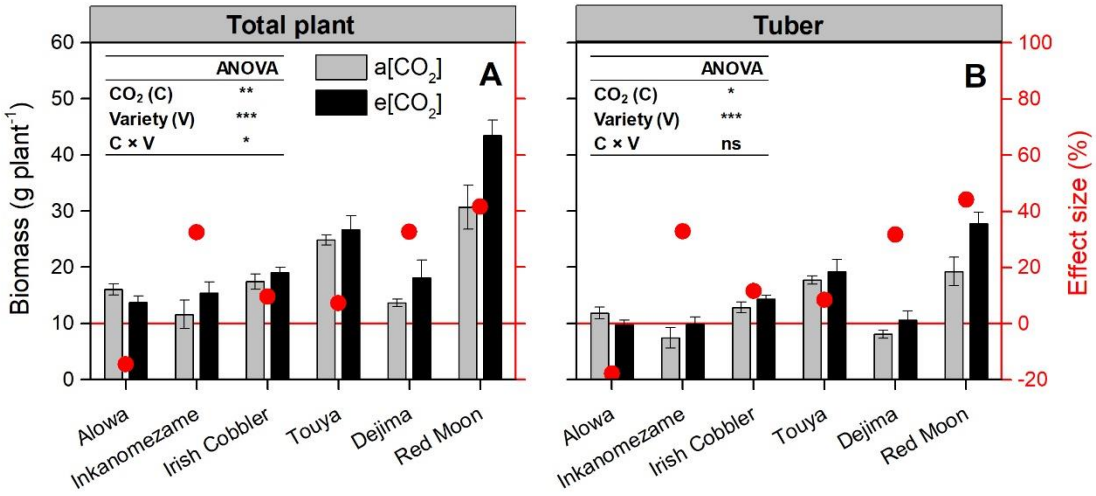

Figure 4.3 (A) Total plant biomass, and **(B)** tuber biomass of potato plants grown under a[CO_2] (414.5 ± 1.6 ppm) and e[CO_2] (750.8 ± 25.3 ppm). Statistical comparisons (two-way ANOVA) between CO_2 (C) and variety (V) as well as their interaction (C × V) are presented. Effect size of e[CO_2] on total plant and tuber biomass were also shown in the corresponding figures. Effect size (%) = (e[CO_2] $_{Biomass}$ − a[CO_2] $_{Biomass}$) × 100%/ a[CO_2] $_{Biomass}$.

Biomass allocation

Biomass allocation was little affected by CO_2 conditions, however varied among varieties (Figure 4.4). Most biomass was allocated to tuber from 59-75%, and then leaf from 19-29%. Biomass allocated to stem and root was small, 5-12 % and 1-2%, respectively. From Figure 4.4, tuber proportion was higher than 70% in Alowa, Irish Cobbler, and Touya, however, lower than 70% in Inkanomezame, Dejima, and Red Moon. The varieties with higher tuber proportion had lower leaf proportion.

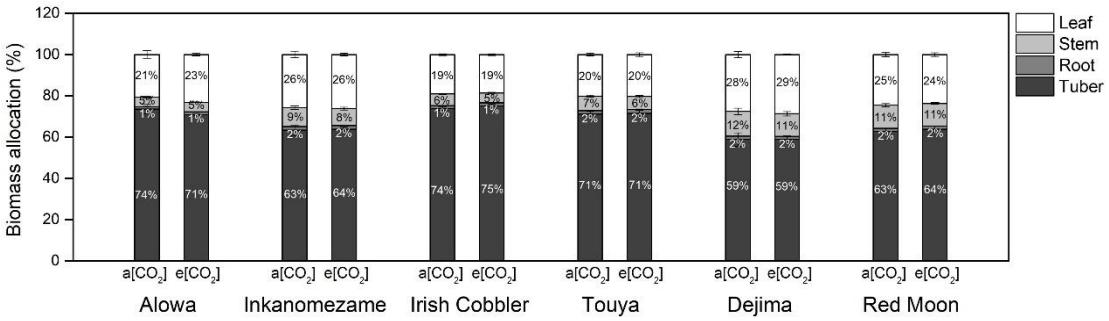

Figure 4.4 Biomass allocation of potato plants grown under a[CO_2] (414.5 ± 1.6 ppm) and e[CO_2] (750.8 ± 25.3 ppm).

Relative growth rate, net assimilation rate, and leaf area ratio

RGR and LAR during the growth period varied among varieties and was larger in medium-late and late varieties than vary early and early varieties (Figure 4.5A and C). NAR was lower in Inkanomezame, Dejima, and Red Moon than in Alowa, Irish Cobbler, and Touya (Figure 4.5B). RGR was strongly associated with LAR, but not with NAR (Figure S4.2).

RGR was increased by e[CO_2] according to ANOVA results (P = 0.025). There was significant interaction between CO_2 and variety on NAR (P = 0.029) and LAR (P = 0.031). The e[CO_2] was likely to decrease NAR in vary early varieties, have no effect on NAR in early varieties, but increase NAR in medium-late and late varieties (Figure 4.5B). LAR was increased by e[CO_2] in Alowa, Inkamomezame, and Dejima, however decreased in Red Moon (Figure 4.5C).

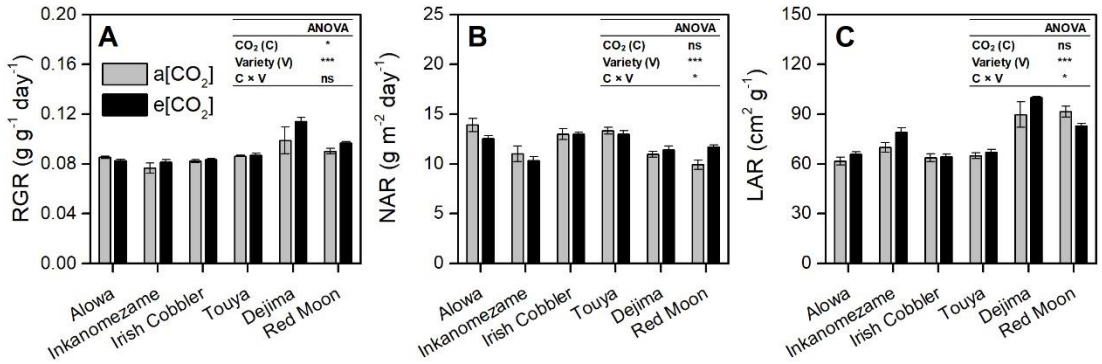

Figure 4.5 (A) Relative growth rate (RGR), **(B)** net assimilation rate (NAR), and **(C)** leaf area ratio **(**LAR) of potato plants grown under a[CO_2] (414.5 ± 1.6 ppm) and e[CO_2] (750.8 ± 25.3 ppm). Statistical comparisons (two-way ANOVA) between CO_2 (C) and variety (V) as well as their interaction (C × V) are presented.

Nutrient statuses in leaves

Leaf [N] was significantly decreased by e[CO_2] and varied a little among varieties (Figure 4.6A). Leaf [P] was similar between a[CO_2] and e[CO_2] but varied in varieties (Figure 4.6B). Leaf C/N was increased by e[CO_2] due to the reduction in leaf [N] (Figure 4.6C). The medium-late and late varieties (Dejima and Red Moon) showed lowest leaf C/N, and the vary early varieties (Alowa and Inkanomezame) showed highest leaf C/N. There was no significant interaction between CO_2 and variety on leaf nutrient statues examined above.

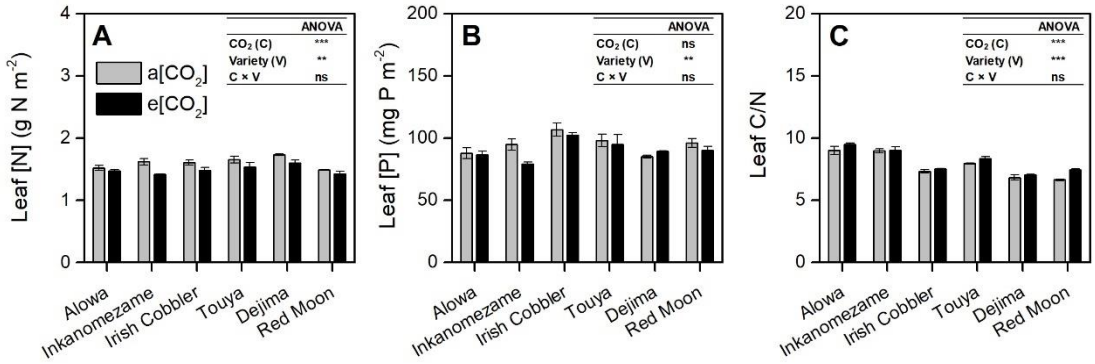

Figure 4.6 (A) Leaf nitrogen concentration ([N]), **(B)** leaf phosphorus concentration ([P]), and **(C)** leaf C/N of potato plants grown under a[CO_2] (414.5 ± 1.6 ppm) and e[CO_2] (750.8 ± 25.3 ppm). Statistical comparisons (two-way ANOVA) between CO_2 (C) and variety (V) as well as their interaction (C × V) are presented.

Nutrient uptake

N and P uptake were different among the varieties (Figure 4.7). P uptake was increased by e[CO_2] (P = 0.028), however N uptake was not affected by e[CO_2] (P = 0.064).

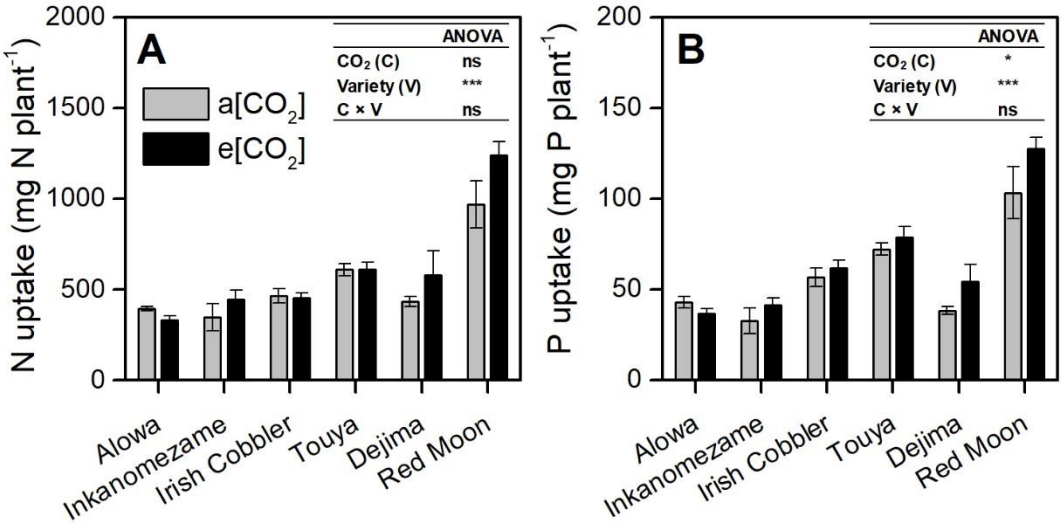

Figure 4.7 (A) N uptake, and **(B)** P uptake of potato plants grown under a[CO_2] (414.5 ± 1.6 ppm) and e[CO_2] (750.8 ± 25.3 ppm). Statistical comparisons (two-way ANOVA) between CO_2 (C) and variety (V) as well as their interaction (C × V) are presented.

Starch, glucose, and sucrose in leaves

Total NSC (starch, glucose, and sucrose) in leaves varied among these varieties (Figure 4.8). The very early varieties, Alowa and Inkanomezame, showed higher starch, glucose, and sucrose comparing with other varieties. The varieties Touya and Dejima showed a similar concentration. Irish Cobbler and Red Moon showed relatively lower concentration. The e[CO_2] significantly increased starch, sucrose, but not glucose.

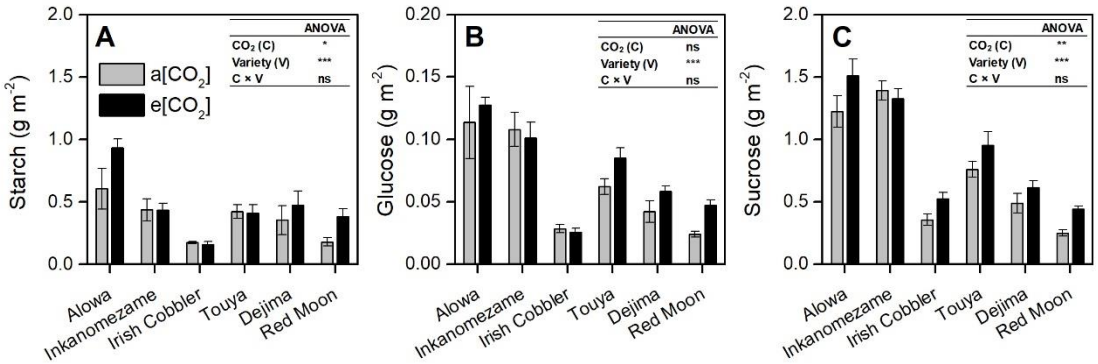

Figure 4.8 (A) Starch, **(B)** sugar, and **(C)** sucrose, in leaves of potato plants grown under a[CO_2] (414.5 ± 1.6 ppm) and e[CO_2] (750.8 ± 25.3 ppm). Statistical comparisons (two-way ANOVA) between CO_2 (C) and variety (V) as well as their interaction (C × V) are presented.

4.4 Discussion

This experiment focused on clarifying whether CO_2 effects depend on variety earliness in potato. Since sink capacity is often thought as a critical trait to determine CO_2 effects, and sink capacity may be limited under deficient nutrient status. Therefore, in this study, sufficient nutrients were supplied to eliminate any potential growth limitation by nutrient deficiency. The reduced leaf [N] under e[CO_2] could be due to dilution effect by the increased biomass. The late variety Red Moon had the highest yield production with highest nutrient uptake (Figures 4.3 and 4.7). Consistent with my hypothesis, the late variety Red Moon had the largest effect size and the very early variety Alowa had the smallest variety.

The late maturity variety may have superiority under an increasing CO_2 condition

The enhancement of total plant biomass by e[CO_2] varied among varieties from -15% to 42% and showed highest value in the late variety Red Moon and lowest value in the very early variety Alowa (Figure 4.3). That was consistent with the hypothesis that CO_2 effects is supposed to be larger in late variety because of its longer life cycle, which may efficiently delay down-regulation of photosynthesis under e[CO_2]. The late variety had a larger effect size, suggesting an importance to maintain vegetative growth under e[CO_2]. However, there is one variety, Inkanomezame, belonging to very early maturing group, showed great effect size of e[CO_2] similar with the late variety, in contradiction with my hypothesis.

A possible explanation for the strong response of Inkanomezame to e[CO_2] may be that it is a diploid variety, which is different from tetraploid of the other five varieties. The diploid variety may have more plasticity to a changing environment. In this study, I hypothesized that CO_2 effect is mainly determined by earliness if accelerated senescence under e[CO_2] is a common phenomenon in potato. Since lifespans of these varieties were failed to be recorded in this study due to early harvest before total

maturity (Figure 4.2A). Further research covering the entire life cycle is needed to verify this hypothesis.

The strategy of resource allocation at early stage seems important for CO_2 effects

Interestingly, I found the varieties (Inkanomezame, Dejima, and Red Moon) with larger leaf proportion had larger effect size on total plant biomass accumulation (Figure 4.3). It may be explained from the following two perspectives, 1) the varieties with larger leaf proportion were on much earlier developmental stage because of their longer vegetative phase, 2) the variety with larger leaf proportion adopt the strategy of increasing source strength first.

For the first perspective, that the varieties, Inkanomezame, Dejima, and Red Moon, had larger leaf proportion at harvest was just because they have longer life cycle. Therefore, they started reproductive growth later than the other varieties. Even the tuber proportion was lower in these varieties at the examined time in this study, it was expected that these varieties can catch up with or even exceed to other varieties at entire maturity. It is easily understood that Dejima and Red Moon had longer life cycle because they are late varieties. The lifespan of Inkanomezame, a very early variety, should be shorter than the late varieties, however, showed lower leaf proportion. Therefore, further research covering the entire growth period is needed.

As for the second perspective, not only sink strength, but also source strength is important for CO_2 effects (Burnett et al., 2016). Generally, crop with large sink capacity is thought to profit more from e[CO_2] (Marschner, 1995). That could be based on the crops with the same source capacity. It is likely that both source and sink capacity are different among these varieties examined in this study. Obviously, that the varieties with higher leaf proportion had larger effect size of e[CO_2] may indicate increase in source first would be benefit more. Therefore, leaf expansion rather than tuber formation at early growth stage may be more important to fully utilize CO_2 from the whole growth period.

What contributed to the larger effect size

Schapendonk et al. (2000) found larger effect size of e[CO_2] on potato in a late variety than an early one. They thought late variety has higher tuber production rates, thus down-regulation of photosynthesis may be less in late variety than early one. However, the very early variety Inkanomezame has a similar effect size with the late variety Red Moon (Figure 4.3A), which make it hard to associate with larger effect size with higher tuber production rate in this study.

Down-regulation of photosynthesis under long-term CO_2 exposure may be related to accumulation of NSC in source leaves because of insufficient sink capacity (Stitt, 1991). In this study, starch and sugar were quietly different among varieties; higher in the very early varieties and lower in the early and late varieties (Figure 4.8). From Figure 4.8, the increase in starch, glucose, and sucrose were highest in Red Moon, however, effect size on biomass accumulation was also largest in this variety (Figure 4.3). There seems to be little relationship between the change of NSC accumulation to e[CO_2] and effect size on biomass accumulation.

To further investigate what contributed to plant growth, I compared RGR, NAR, and LAR during the growth period, which can help understanding the adaptation of plants to specific environmental conditions (Ryser and Wahl, 2001). RGR was increased by e[CO_2] and varied in these varieties (Figure 4.5A). The overall results show that RGR was associated to LAR rather than NAR (Figure S4.2). However, larger RGR under e[CO_2] for each variety was attributed to NAR or LAR. For example, for the vary early variety Inkanomezame, NAR was lower under e[CO_2], thus increased LAR contributed to higher RGR; as for the late variety, LAR was decreased under e[CO_2], thus larger RGR under e[CO_2] was due to higher NAR. From these results, it seems CO_2 effects is limited by NAR in the vary early varieties but LAR in the late variety. That may be associated with e[CO_2]-mediated senescence, thus early decrease of assimilation rate under e[CO_2] in the vary early varieties.

4.5 Conclusions

This study aimed to clarify the variety earliness on CO_2 effects in potato plants. Six potato varieties belonging to distinct maturing groups were examined in this study. The e[CO_2] increased tuber and total plant biomass in all varieties except for the very early variety Alowa. The late variety Red Moon likely demonstrates greatest CO_2 effect, suggesting an importance to resist senescence under e[CO_2]. Additionally, the varieties (Inkanomezame, Dejima and Red Moon) with higher leaf but lower tuber proportion was found having greater effect sizes of e[CO_2] in total plant biomass comparing to the others. That indicates leaf expansion rather than tuber formation at early growth stage may be more important to fully utilize CO_2 from whole growth period. Lower NAR under e[CO_2] in very early varieties, but higher NAR under e[CO_2] in late varieties indicates the possibility of e[CO_2]-mediated senescence is dependent on earliness of potato plants. Since plants were harvested before total maturity, further study is needed to confirm clear senescence of plant and effect size of e[CO_2].

Figure S4.1

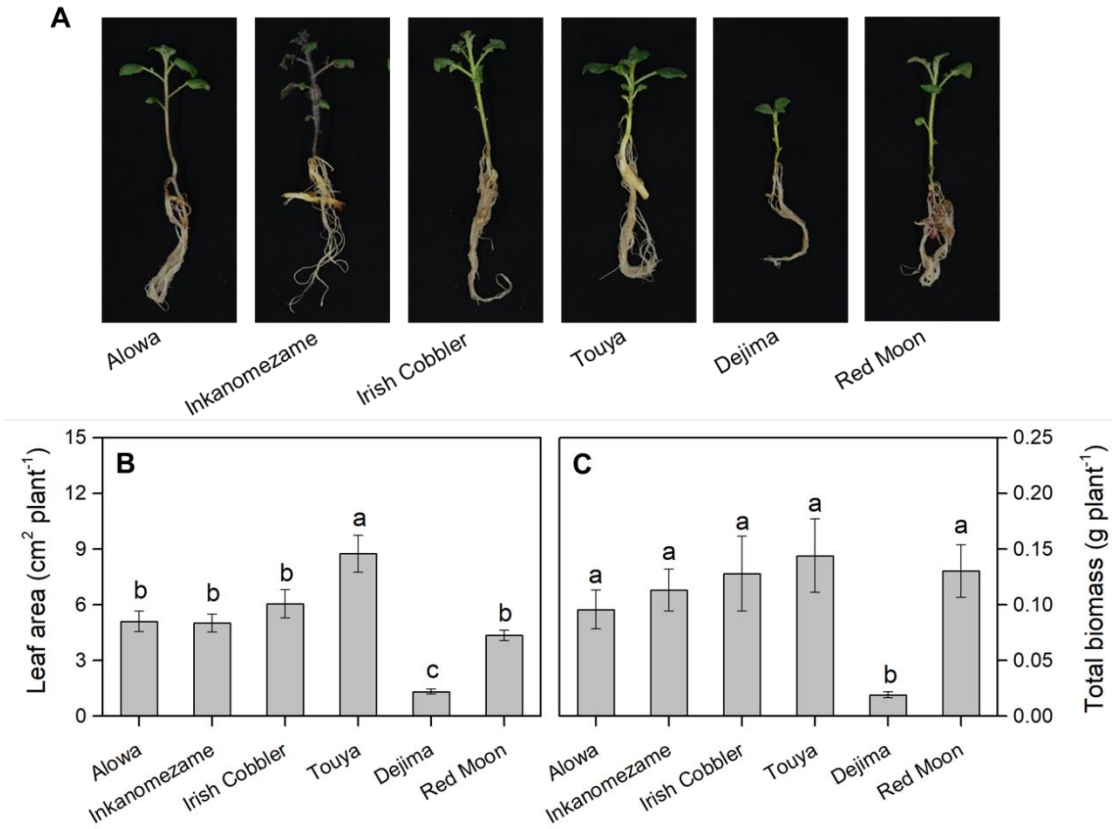

Figure S4.1 (A) Plant appearance at transplanting. **(B)** Leaf area, and **(C)** total plant biomass of different varieties of potato plants at transplanting. Data are means ± SE of four biological replicates. Bars marked with the same letter did not significantly different at the 0.05 probability level.

Figure S4.2

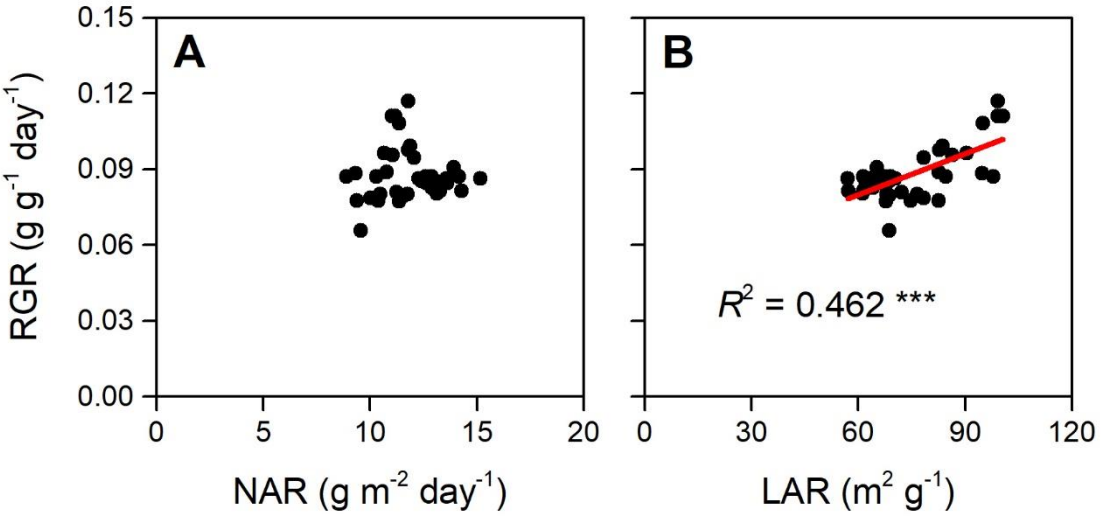

Figure S4.2 (A) Relation between relative growth rate (RGR) and net assimilation rate (NAR). **(B)** Relation between RGR and leaf area ratio (LAR).

Chapter 5

General discussion and conclusions

5.1 General discussion

e[CO_2] enhanced maximum plant growth without additional phosphorus, but with additional nitrogen

Similar with previous studies in potato (Fleisher et al., 2012, 2013b), growth promotion by e[CO_2] was dependent on nutrient supply in this study (Figures 2.1.3 and 2.2.4). In previous studies, CO_2-fertilization effects on potato plants have been examined at only three levels of nutrient supply (Fleisher et al., 2012, 2013b), thus being indifferent to maximum growth and its saturation. In this study, five or six nutrient supply rates were set to get the response curve of plants to e[CO_2], thus quantifying the minimum nutrients and water demands for the maximum plant growth. Additionally, critical nutrient concentration, defined as the minimum nutrient requirement for 90% maximum plant growth was estimated to verify our conclusion about nutrients demands under e[CO_2]. The results shows that to reach maximum plant growth under e[CO_2] at early developmental stage, additional P supply is not needed, however, more N supply is required (Figures 2.1.3, 2.1.9, 2.2.4, and 2.2.13). To fully profit from an increasing CO_2, sufficient nutrient is required. However, excessive nutrient supply causes waste of fertilizer and even environment pollution. Therefore, quantifying nutrient demand for the maximum plant growth under e[CO_2] could provide an important instruction for fertilizer management in potato plants under an increasing atmospheric CO_2 concentration in the future.

Plant growth was enhanced by e[CO_2] without additional water consumption due to improved water-use efficiency

In this study, WUE at plant level (dry matter production per water consumption) was estimated (Figures 2.1.7, 2.2.8, and 2.3.7), which is more informative for agricultural purpose, than instantaneous or intrinsic WUE at leaf level (Sinclair et al., 1984). To reach the maximum plant growth, no additional water was consumed (Figures 2.1.7 and 2.2.8). Though the maximum plant growth in response to K supply was not obtained

(Figure 2.3.3), water use at each K supply was decreased by e[CO_2] under HP (Figure 2.3.7B). Plant growth was enhanced by e[CO_2] without additional water consumption due to improved WUE, which is further affected by nutrient supply (Figures 2.1.7, 2.2.8, and 2.3.7). Crop under e[CO_2] with suitable nutrient supply is expected to use water economically. This finding could make great significance for crop production under e[CO_2] in the future, especially in arid areas, as approximately 70% of the fresh water is used in agriculture in the world (Clarke and King, 2004).

The e[CO_2] accelerated plant growth through the life cycle

Accelerated senescence under e[CO_2] was clearly observed in this study (Figures 3.1 and 3.5), which have also been reported in some previous studies (Bindi et al., 2006; de la Mata et al., 2012). The effects of e[CO_2]-induced accelerated senescence on plant biomass accumulation varied in this study; growth was promoted by e[CO_2] even senescence was accelerated under sufficient nutrient conditions in GC (Figure 3.1), however, biomass was decreased by e[CO_2] in OTC due to earlier senescence even under sufficient nutrient conditions (Figure 3.5). Generally, productivity is related to leaf area duration for tuber crops (Gregersen et al., 2013), indicating accelerated senescence may reduce productivity. That e[CO_2] enhanced plant growth with accelerated senescence could be of great importance for agricultural production, because higher crop production can be achieved with short growth period. Other merits from the reduced crop duration have been pointed out by Miglietta et al. (1998), such as savings in water consumption and in manpower for cultivation.

Improving crop production by delaying e[CO_2]-induced senescence

A critical problem is that e[CO_2]-induced senescence could also have negative impacts on crop production under unfavorable conditions, such as nutrient deficiency. Figure 3.5 showed reduced biomass by e[CO_2] due to accelerated senescence. That may be related to the short lifespans of plants in OTC, where growth conditions, such as irradiance, were quite different with that in GC. Miller et al. (1997) suggested that lower

photosynthetic rate after long-term exposure to e[CO_2] is the result of earlier shift in the timing of the normal photosynthetic stages of leaf ontogeny associated with senescence. That may be why growth increment under e[CO_2] have always been observed lower at the end of growing season than the intermediate harvest (Craigon et al., 2002).

According to the possible shift model of photosynthetic rate in Figure 5.1, the effects of e[CO_2] on plant biomass accumulation during the whole growth period is dependent on both the growth promotion during early stage and adverse effects during late stage. Under unfavorable growth conditions, such as nutrient deficiency, plant growth at early stage cannot be promoted by e[CO_2], eventually result in poor biomass accumulation at the end of growing season due to accelerated senescence under e[CO_2] (Figure 5.1A). To fully benefit from e[CO_2], efficient approaches are required, such as fertilizer management and variety breeding. Sufficient nutrient supply, especially at early developmental stage, could allow potential photosynthetic rate be achieved and accumulate more biomass under e[CO_2] (Figure 5.1B). Preferable variety, such as late maturing variety, may have longer vegetative growth period, thus having more advantage in time to accumulate biomass when photosynthetic rate is higher under e[CO_2] than a[CO_2] (Figure 5.1C).

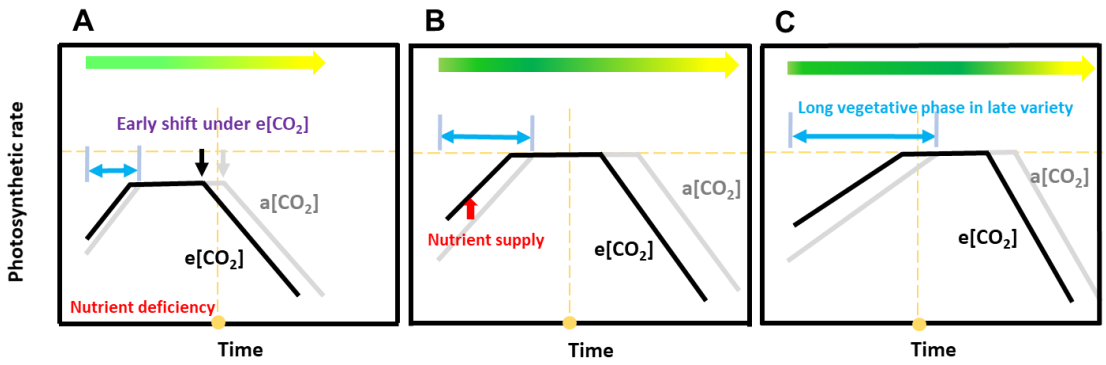

Figure 5.1 A diagram illustrating shift model of photosynthetic rate during the life under a[CO_2] and e[CO_2].

5.2 Conclusions and prospect

Potato plant growth could be promoted by e[CO_2], however, the enhancement was dependent on nutrient supply. To reach the maximum plant growth under e[CO_2] at early growth stage, P supply was not increased but N supply was increased. Considering early senescence under e[CO_2], nutrient demand at late developmental stage may be affected. Thus, further study focusing on nutrient demand at late stage is needed. Additionally, effects of e[CO_2] in GC was inconsistent with that in OTC, which may be due to the altered lifespans. Lifespan of a specific species can be affected by environment factors and genotype difference. Nutrient supply and variety selection were suggested to be efficient approaches to improve CO_2 effects in this study. This study focused on the potato responses to climate change of e[CO_2], however, the increase of CO_2 concentration in atmosphere is often accompanied with other climate changes, such as increased temperature and drought, which are supposed to impact plant growth under e[CO_2]. Therefore, further study on effects of nutrient supply on potato growth at e[CO_2] should be considered to combine with other climate factors.

Appendix: description of measurements

Measurement of water use (Chapter 2)

A transparent plastic film was used to cover each pot to prevent water loss by soil evaporation. Because the pots used had no holes in the bottom, leaching was not considered. I weighed the pots until harvest before watering. A decrease in pot weight was regarded as water consumption by transpiration, and the same amount of water lost by transpiration was provided to each pot. Considering plant keep growing along with the time, extra water was provided to keep soil water content at the specific range. The pot weight and the amount of water given to each pot were recorded throughout the growth period. Water use during the growth period was calculated from cumulative transpiration. WUE was calculated as total plant biomass/ water use according to Jones (2004).

Nitrogen determinations in plant material (Chapter 2, 3, and 4)

Approximately 1.3-1.7 mg of ground dried samples were encapsulated in 0.15-ml tin foil and processed through an elemental analyzer (Vario EL, Elementar Analysensysteme GmbH, Hanau, Germany).

OR

Approximately 3 mg of ground dried samples were encapsulated in 0.15-ml tin foil and processed through an elemental analyzer (FLASH 2000, Thermo Fisher Scientific, Worcester, MA, USA).

Phosphorus determination in plant material (Chapter 2, 3, and 4)

P concentration was determined according to Watanabe and Olsen (1965). Dried plant samples (40−60 mg) were weighed into crucibles and ashed at 495 °C for 2 h. After cooling to room temperature, 1 mL 4 M HCl was added to the crucibles and then transferred to 25-mL volumetric flasks. The distilled water used to wash the crucibles

for three times was also transferred to the same volumetric flasks, and identical volumes across flasks were obtained by adding distilled water. An appropriate amount of sample solution was transferred to a new 25-mL volumetric flask, and 4 mL color-substrate solution (2.5 M H_2SO_4 : 4% $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$: 0.1 M $C_6H_8O_6$: 4.4 mM $C_8H_4K_2O_{12}Sb_2$ = 10 : 3 : 6 : 1) was added first, followed by distilled water to constant volume. The solutions were mixed and incubated at room temperature for 15 min; next, absorbance at 710 nm (A_{710}) was measured using a UV spectrophotometer (UV-1800, Shimadzu Inc., Japan).

Potassium determinations in plant material (Chapter 2 and 3)

Approximately 0.2 g ground dried plant samples were weighted into 50-mL bottles. Next, 15 mL 1 M HCl was added to each bottle. Extracts were filtered through an ashless paper after oscillation for 30 min and extraction for 24 h. The filtrates were used to measure K in plant material by using a digital inflammatory photometer (ANA-135, Tokyo Photoelectric Co., Ltd., Japan).

Non-structural carbohydrate quantification (Chapter 2, 3, and 4)

Starch, glucose, and sucrose content were determined after Ono et al. (1996). Samples (3-5 mg each) of micro-ground dried tissue were placed in 2-mL microtubes containing 0.75 mL 80% ethanol and heated at 78.5 °C for 10 min on a heating block. The supernatants were collected for glucose and sucrose quantification after centrifugation (12,000 ×g, room temperature, 10 min) and 0.5 mL 80% ethanol was added into each tube to heat at 78.5 °C for 10 min once again. After centrifugation at 18,000 ×g, at room temperature for 10 min, the supernatants were also collected and residues containing starch were dissolved in 400 µL Milli-Q water, heated at 98 °C for 1 h, and then cooled to room temperature. After adding 400 µL amyloglucosidase (70 units G-Amyloglucosidase/mL 50 mM Na-acetate buffer at pH 4.5), samples were incubated at 55 °C for 1 h. After digestion of starch to glucose, samples were centrifuged at 18,000 ×g, at room temperature for 10 min, and the supernatants were then assayed for glucose

using a Glucose CII test kit (Wako Chemicals, Tokyo, Japan). The assay reagents were mixed into the samples and the reaction was incubated for 10 min at room temperature before measuring their absorption at 505 nm (A_{505}) in a microplate reader (Sunrise Rainbow Thermo, Tecan Japan, Co., Ltd., Japan).

The supernatants collected above were used to quantify glucose and sucrose. The supernatant was evaporated to remove ethanol with a centrifugal concentrator (5301, Eppendorf, Hamburg, Germany). The same volumes of Milli-Q water and chloroform were added to the concentrated supernatant. After centrifugation at 12,000 ×g, at room temperature for 10 min, the upper clear phase was collected for glucose analysis. Sucrose in the solution was hydrolyzed with invertase at room temperature for 1 h, and then determined from the difference between total glucose and free glucose.

Soil available P after harvest (Chapter 2)

Dried, 1-g soil samples were weighed into 200-mL bottles. Next, 200 mL 1 mM H_2SO_4 (pH 3.0) was added to each bottle. Extracts were filtered through an ashless paper after oscillation for 30 min. The filtrates were used to measure available P according to the method described above for P quantification in plant materials.

Soil pH after harvesting (Chapter 2)

Dried, 10-g soil samples were weighed into 50-mL tubes; next, 25 mL distilled water was added to each tube. After vigorous stirring, tubes were allowed to stand for 30 min. A pH meter (LAQUAact D-73, HORIBA Inc, Japan) was used to measure the pH of the soil solution.

SPAD measurement (Chapter 3)

SPAD was measured by using a SPAD portable meter (SPAD-502, Konica Minolta Sensing, Japan). Five measurements were made on each leaf.

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